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CLINICAL AND EXPERIMENTAL

STUDIES ON THE EXCRETION OF CHLORIDE BY MAN WITH AND WITHOUT CONGESTIVE HEART FAILURE, USING LONG-LIFE RADIOCHLORIDE, Cl^{36}

C. T. RAY, M.D., S. A. THREEFOOT, M.D., AND G. E. BURCH, M.D.
NEW ORLEANS, LA.

STUDIES in which the long-life radiochloride, Cl^{36}, was employed to investigate the biologic decay rates of chloride in man with and without congestive heart failure[1] were designed to take advantage of the long-life tracer to make it possible to investigate aspects of the excretion of chloride not amenable to investigation with the short-life isotope, Cl^{38}. Among the problems which needed clarification and which were approached in these experiments was definition of the time course of excretion of a single dose of radiochloride by essentially normal man and those suffering from a disease known to alter chloride excretion. This is important for a better understanding not only of electrolyte metabolism but also of the problems of injury consequent to internal exposure to radiochloride. Data are presented which indicate the influence of various factors upon the rate of excretion.

Analysis of the data includes correlation of the excretion and clearance rates for Cl^{36}, Cl^{35}, sodium, potassium, and water over long periods of time for the normal physiologic state; variations in dietary intake of certain electrolytes; variations in the state of congestive heart failure; and therapeutic procedures

From the Department of Medicine, Tulane University School of Medicine, and Charity Hospital of Louisiana at New Orleans.

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TABLE I. CLINICAL DATA AND EXCRETION OF Cl^{36} DURING FIRST 90 MINUTES

SUBJECT	INJECTED DOSAGE (CPM)	DURATION OF STUDY (DAYS)	AGE (YEARS)	INITIAL WEIGHT (KG.)	DIAGNOSIS	TREATMENT	APPEARANCE IN URINE (MIN.:SEC.)	TOTAL Cl^{36} EXCRETED 90 MIN.		TOTAL URINE VOL. (ML.)	AV. URINE FLOW (ML./MIN.)
								CPM	% DOSAGE		
501	10^7	69	31	47.27	Thyrotoxicosis	Regulated with propylthiouracil	1:00 to 7:00	34634	0.346	33.5	0.37
504	1.02×10^7	47	44	50.34	Tuberculous lymphadenitis	Streptomycin	2:10 to 2:30	39632	0.388	156.7	1.74
502	(?) 10^7	70	62	40.91	Diabetes; hypertensive cardiovascular disease; mild congestive heart failure	Digitalis	4:55 to 7:05	61610	0.616	283.3	3.15
505	1.25×10^7	72	50	64.77	Arteriosclerotic heart disease; moderate congestive heart failure; edema marked	Digitalis	4:30 to 5:50	28949	0.232	79.2	0.88
503	10^7	37	59	67.27	Arteriosclerotic heart disease; severe congestive heart failure; edema marked; expired	Digitalis	Indeterminable	240	0.002	22.2	0.25
506	10^7	32	54	46.13	Hypertensive and arteriosclerotic heart disease; aortic insufficiency; pulmonary emboli and infarction; edema marked; expired	Digitalis	0:00 to 3:00	2100	0.021	79.5	0.88

employed in the treatment of congestive heart failure, as well as other factors. In addition to providing information from studies extending over long periods of time, by proper design of the experiments it was also possible to investigate the more rapid and earlier excretory phenomena concerned with the elimination of the tracer by man, including the time of appearance of the tracer in the bladder urine following its intravenous administration and the time course of excretion of the radiochloride during the first few hours. The relation of the excretion of chloride to estimation of chloride space and exchanging mass was approachable by the use of the long-life tracer. This phase of the investigation was particularly important in view of previously described investigations related to chloride space and mass.[2, 3] It was possible to define some of the marked variations in excretion and their influence upon the calculations of space and mass by the tracer method.

MATERIALS AND METHODS

Radiochloride, approximately ten million CPM ($\simeq$ 50 μc) as $NaCl^{36}$, was administered intravenously to two control subjects and four subjects with chronic congestive heart failure (Table I). The exact dosage administered was known in all subjects with the possible exception of Subject 502. Samples of venous blood were collected rapidly at intervals of a few seconds for the first few minutes and the intervals progressively lengthened to 15 minutes by the end of two hours. From the end of the second hour to the end of the experimental period, 30 to 70 days, blood samples were collected each morning except Sunday and on occasion when, for special purposes, they were collected at shorter intervals. During the first two hours following the administration of the isotope, urine was collected from a catheter in the bladder as often as possible and as nearly coincident with the collection of the blood sample as the rate of flow of urine would permit. From the end of the second hour to the end of the study period, each voided specimen of urine was collected in a separate bottle except when special study warranted collections at specific intervals of time. All other body fluids and excreta also were collected separately for the entire period of study.

The dietary intake of sodium, chloride, potassium, and water* was calculated from tables.[4] The food was weighed before and after meals. A special attendant was constantly present to assure the accuracy of the dietary intake and collection of all excreta.

Samples of serum, urine, and body fluids (ascitic and pleural) were assayed for sodium and potassium with a Beckman flame photometer, making suitable corrections for mutual excitation by means of calibration data for this particular instrument.[5] Chloride was measured by the method of Schales and Schales[6] and radiochloride determined with a thin-mica-window Geiger-Müller tube, the samples having been prepared for counting by a method previuosly described.[1, 7, 8] Stools were assayed only for radiochloride. Radiochloride was determined in every sample of urine; except in special instances, chloride, sodium, and potassium were determined only in the pooled 24-hour sample from 9 A.M. to 9 A.M., the time at which the daily samples of blood were also drawn.

During the periods of observation, special investigations were conducted which included a study of the effects of the administration of a mercurial diuretic, infusions of sodium chloride, and administration of various drugs, such as desoxycorticosterone acetate, potassium acetate, and sodium bromide. On some of these occasions the concentrations of all three electrolytes in each sample of urine were determined and additional samples of blood were drawn for assay.

RESULTS

For convenience of presentation the data are divided into four parts: (1) excretion of Cl^{36} during the first 90 minutes following the intravenous

*For these studies, the cooperation of Sister Euphemia and Misses Gloria Kicklighter and Jeanne Reid of the Dietary Department of Charity Hospital is greatly appreciated.

administration of a single dose of the tracer; (2) recovery of the isotopes by various routes of excretion; (3) daily excretion, clearance, and balance of Cl^{36}, Cl^{35}, sodium, and potassium; and (4) influence of special procedures upon the excretion of chloride.

1. *Excretion of Cl^{36} During the First Ninety Minutes Following the Intravenous Administration of a Single Dose of the Tracer.*—

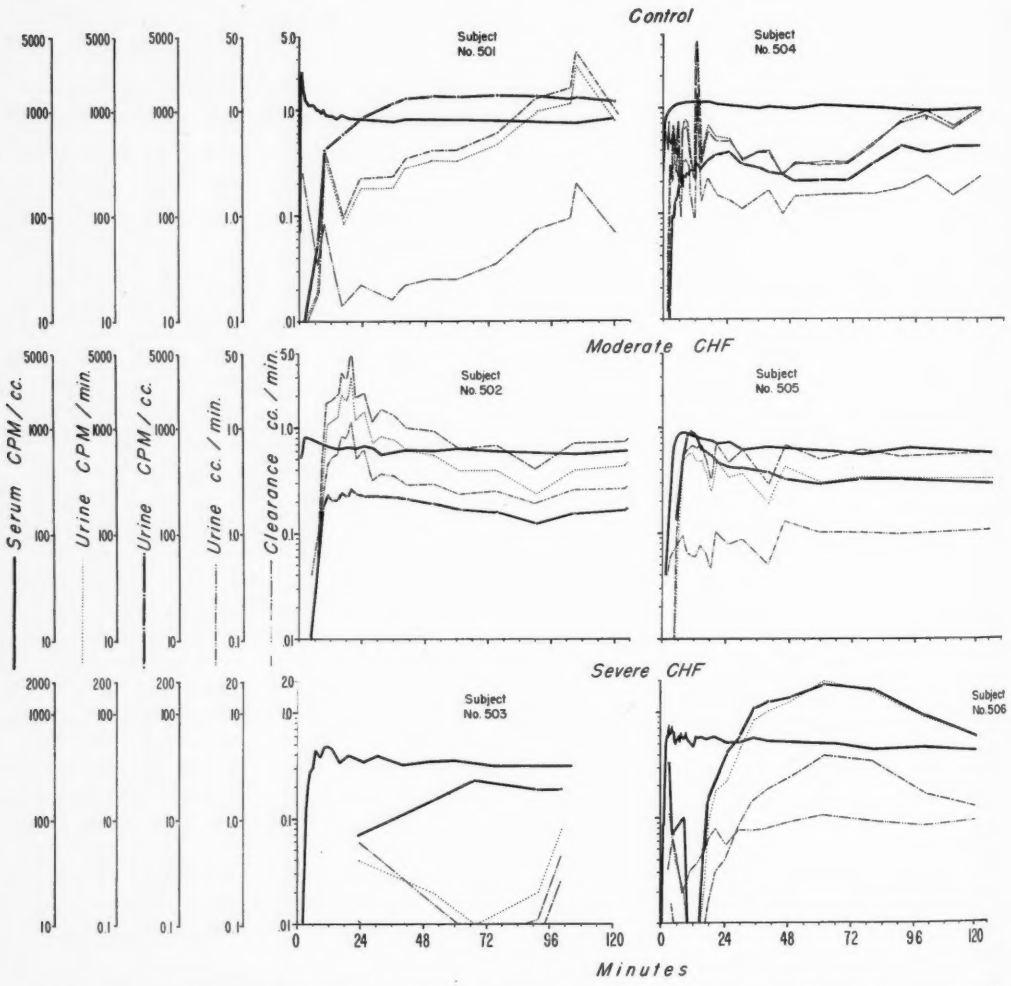

Fig. 1.—Concentration time course of Cl^{36} in serum, urinary concentration, rate of urinary excretion, rate of urine flow, and urinary clearance of Cl^{36} in the six subjects studied (Table I) for the first two hours following intravenous administration. The ordinate values for rate of urinary excretion and urinary concentration for the subjects with severe congestive heart failure are 1/100 those of the other two groups.

A. *The time of appearance of the tracer in the urine:* Because of technical difficulties and failure to collect sufficient quantities of urine for counting from some patients, it was not possible to determine the precise time at which the radiochloride first appeared in the urine, but only that time interval during which it appeared (Table I and Fig. 1). In one subject (Subject 504) the

time of the first appearance of the Cl^{36} in the bladder urine was between 2 minutes 10 seconds and 2 minutes 30 seconds, the appearance being most precisely obtained in this patient because of short collection intervals. Although determined less precisely (within 1- to 2-minute intervals) in Subject 502, the time of appearance was between 4 minutes 55 seconds and 7 minutes 5 seconds, and in Subject 505, between 4 minutes 3 seconds and 5 minutes 50 seconds. In Subject 501 radiochloride appeared in the urine between 1 and 7 minutes and in Subject 506, some time before 3 minutes had elapsed. The concentration of Cl^{36} in the urine of Subject 503 with severe congestive heart failure was so low that it was not possible to distinguish between significant excretion of the tracer and fluctuations in background. From these data there was no detectable difference between the time of appearance of the tracer in the urine in the control subjects and the subjects with congestive heart failure. Every subject for whom it was possible to measure significant quantities of tracer in the urine had excreted some of the tracer by 7 minutes after the intravenous administration. These results are similar to those previously reported for Na^{24}.[9]

Comment: Although it was realized that the use of bladder catheters would not permit determination of the precise time at which the tracer entered the bladder urine, this collection technique was employed in an attempt to measure as closely as possible the time of appearance of Cl^{36} in the bladder. Some of the difficulties included rates of urine flow inadequate to permit frequent collections of volumes large enough for study, inability to exclude pooling in the bladder before entering the catheter, and the time required for transit through the catheter. The catheters employed had a luminal volume of approximately 2 ml. so that at rates of urine flow of 1 ml. per minute, two minutes would have been required for transit through the catheter. Furthermore, the curve of excretion of the isotope progressed so gradually from zero that it was difficult to determine precisely when the counts became significantly greater than background.

It was noted that psychological disturbances associated with the studies most probably produced considerable variations in the rate of urinary flow, for subjects who seemed to be frightened by the activity attendant to the rapid collection of samples developed anuria lasting several minutes. The degree to which such disturbances influenced measurements of the appearance time, as well as other phases of these studies, has not been determined.

As pointed out in the studies with radiomercury,[10] it was not even possible to relate precisely the electrolyte content of a given sample of urine collected from a ureteral catheter to that of a sample of blood collected at the same time from a catheter in a renal vein. Because this lag was not constant, it would be precarious to estimate renal function from clearance data obtained when a lag of this nature is significant, particularly when the concentration of the substance measured changed rapidly in either the blood or urine. Therefore, because the fluctuations in both time of appearance and in rates of excretion may be considerable from individual to individual as well as from time to time

in the same individual, it is not possible to employ a given correction for the lag in appearance time. This problem has been presented in more detail previously.[10]

B. *The rates of urinary excretion of the tracer:* It is apparent from Table I and Fig. 1 that the rate of excretion of chloride in the initial 90-minute period was less for the subjects with edema (Subjects 505, 503, and 506) than for the subjects without edema. In spite of the low rate of urine flow, mean of 0.37 ml./min., for control Subject 501 the rate of output of Cl^{36} for the 90 minutes was relatively high, 385 CPM/min. Subject 502 with mild congestive heart failure who was recovering from the failure at the time of observation had both a urinary flow, 3.15 ml./min., and rate of output of Cl^{36}, 685 CPM/min., which exceeded that of the control subjects. Subjects 503 and 506, who had severe congestive heart failure, showed the least rapid rates of excretion of Cl^{36}, 3 and 23 CPM/min. respectively. Subject 503 had a low rate of urinary flow, 0.25 ml./min., whereas Subject 506 had a fairly high rate of flow, 0.88 ml./min. During a portion of the two-hour period following the intravenous administration of the tracer, the urinary volume declined and the concentration of the tracer rose in Subjects 501, 504, 503, and 506. This temporary inverse relationship had been noted previously for radiomercury.[10]

The time course of urinary *clearance* of the Cl^{36} for the six subjects studied during the first two hours of observation is indicated by Fig. 1. The clearance rates of Cl^{36} were considerably lower in the subjects with severe congestive heart failure than in the control subjects. The clearance rates of the subject with mild congestive heart failure (Subject 502), who was recovering during the period of the study, exceeded those of the control subjects. The time course curves of urinary clearance and the rate of excretion of the isotope and the rate of excretion of water were essentially parallel in all subjects (Fig. 1).

Because of the arbitrary mathematical calculation of clearance, and the lag between change in blood concentration and the appearance of the tracer in a sample of urine produced from a given concentration in the plasma, the clearance values are subject to considerable error, especially during the first ten minutes. When the serum concentration was not changing and therefore became a constant in the clearance ratios, the rate of excretion of the isotope provided the same information concerning the chloride excretory function of the kidney as did the clearance.

The time course of the excretion of radiochloride per minute expressed as a percentage of that present in the body during the first two hours, as well as the next five days, and the ninth day of the experiment, is shown in Fig. 2. When expressed in these units, the rate of excretion of chloride was considerably less in the subjects with severe congestive heart failure than in the two control subjects and the subject with mild congestive heart failure who was recovering. The rate of excretion, expressed as a percentage of the isotope in the body remaining to be excreted, varied considerably from time to time including diurnal variations.

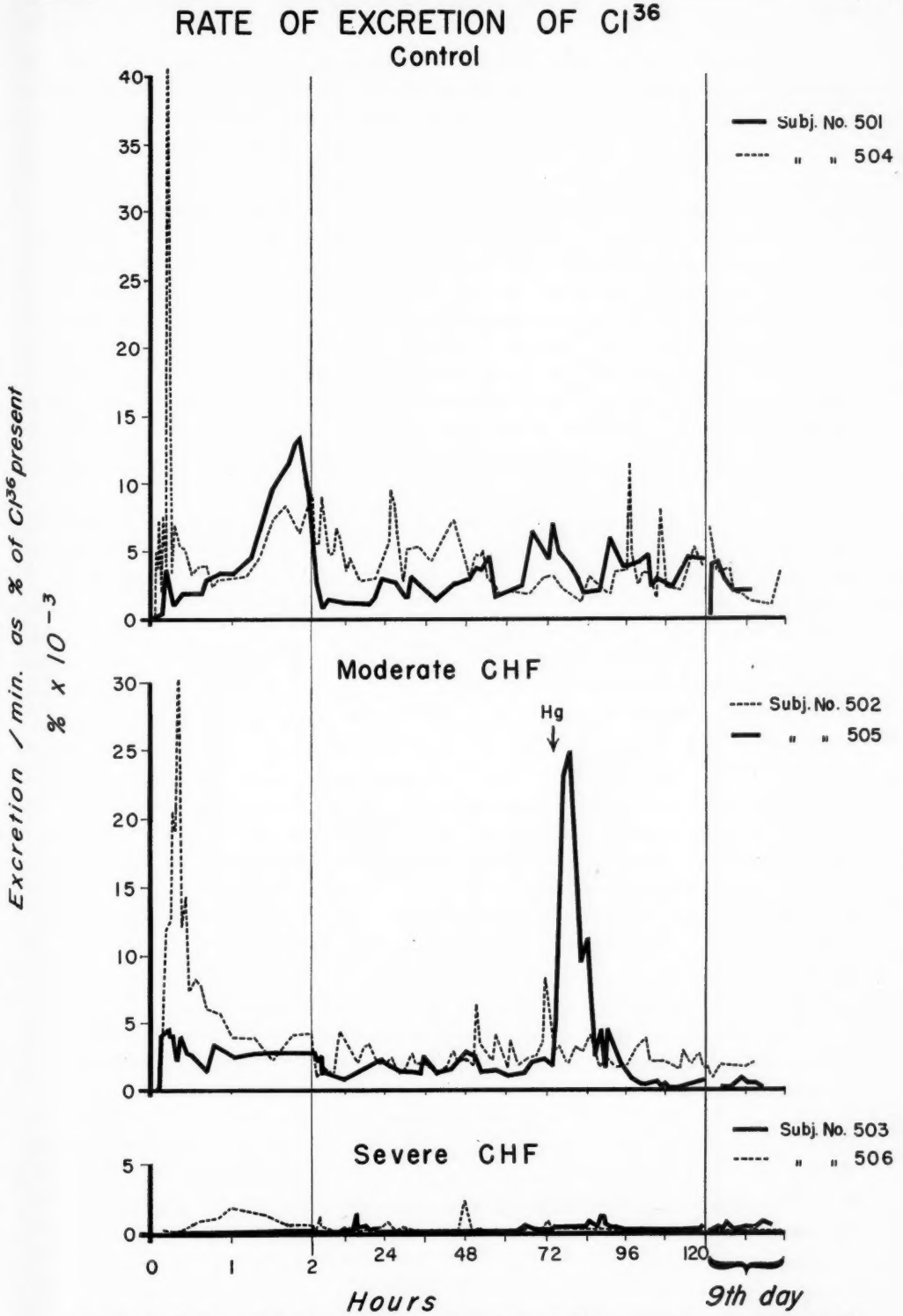

Fig. 2.—Rate of excretion of Cl^{36} as a percentage of that present in the body to be excreted for the first five days and for the ninth day following intravenous administration of the tracer in all six subjects. The two subjects with severe congestive heart failure excreted an extremely small amount of Cl^{36}. The sudden and marked increase in the rate of excretion of Cl^{36} on the third day in Subject 505 was produced by a mercurial diuretic.

Comment: These data present additional evidence of the inability of subjects with congestive heart failure to excrete chloride as compared to normal subjects. Furthermore, when congestive heart failure begins to subside, the excretion of chloride progressively returns toward normal and may actually exceed the rate of excretion of the control subjects on a comparable dietary regimen. These data show this phenomenon in greater detail than has been previously demonstrated because the use of the isotope made possible the study of many samples at frequent intervals on small aliquots of biologic fluids not as readily amenable to the study of Cl^{35} by chemical methods. The time-course curves concerned with the excretory function of the kidney as far as chloride is concerned revealed the marked fluctuation in this function. In fact, in spite of the low rates of excretion in those subjects with impaired cardiac reserve, the percentile variations were of the same magnitude as for subjects with unimpaired cardiac reserve. These low rates of excretion, illustrated by Table I and Figs. 1 and 2, are responsible for the low biologic decay rates previously described.[1]

The similarity of the decrease in the rate of urinary flow simultaneously with an increase in concentration of the tracer in the urine during the period shortly after the injection of the material containing the tracer to that observed following the intravenous administration of radiomercury[10] suggested the possibility of a common mechanism for both. It was thought previously that mercury had some pharmacologic action upon renal function which might have been responsible for the relative characteristics of these two curves when that material was employed. Since the same phenomenon was observed for $NaCl^{36}$ in water, that mechanism of the reaction becomes less likely and a mechanism which might possibly be common to both was suggested. This may consist of the psychological disturbances, associated with the conduct of the experiment or the injection of material with properties foreign to the circulation, either physical or chemical. Its action could be mediated through the central nervous system or by humoral mechanisms.

As would be expected from the low excretion rate of Cl^{36} in the subjects with congestive heart failure, the total amount of tracer excreted during the first ninety minutes, expressed as a percentage of the injected dose, was considerably less than that excreted by the controls (Table I).

The rate of excretion of Cl^{36} expressed as a percentage of that present in the body to be excreted for the first ninety minutes following injection was plotted against body weight of the subject. It was found that the rate of excretion was slower in patients with severe congestive heart failure than those with mild failure (Fig. 3). This is most likely the result of the renal function. Other factors, such as mass of nontracer available for excretion, could have played a role in this relationship,[1, 2, 3, 11, 12] but body weight by itself is not necessarily an index of the mass of nontracer available for excretion. This plot (Fig. 3) suggests the feasibility of the rate of excretion of a test dose of Cl^{36} as an index of the functional state of the kidneys and the state of congestive heart failure. Incidentally, the rate of excretion for Na^{24} was the same as for Cl^{36} in subjects with the same clinical states.[9]

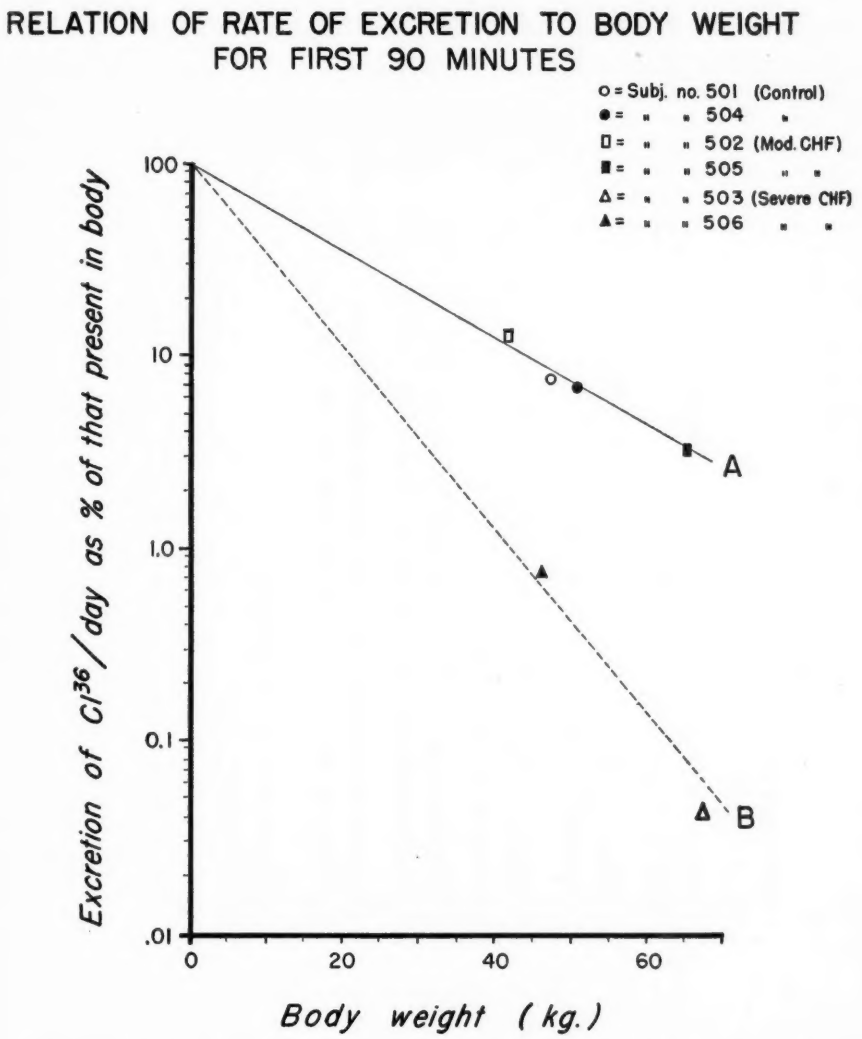

Fig. 3.—Average rate of excretion of Cl^{36} expressed as excretion per day as a percentage of the Cl^{36} present in the body related to body weight for the first ninety minutes following intravenous administration of the tracer. The apparent grouping of values around two separate lines is determined by the ability to excrete chloride rather than by the total body weight. The two subjects with severe failure were able to excrete very little Cl^{36}.

TABLE II

SUBJECT	INJECTED DOSAGE (CPM)	30 DAY RECOVERY							DURATION OF STUDY (DAYS)	TOTAL EXPERIMENTAL RECOVERY						
		ALL ROUTES	URINARY		FECAL		OTHER			ALL ROUTES	URINARY		FECAL		OTHER	
		PER CENT OF INJECTED DOSAGE	PER CENT OF INJECTED DOSAGE	PER CENT OF RECOVERED	PER CENT OF INJECTED DOSAGE	PER CENT OF RECOVERED	PER CENT OF INJECTED DOSAGE	PER CENT OF RECOVERED		PER CENT OF INJECTED DOSAGE	PER CENT OF INJECTED DOSAGE	PER CENT OF RECOVERED	PER CENT OF INJECTED DOSAGE	PER CENT OF RECOVERED	PER CENT OF INJECTED DOSAGE	PER CENT OF RECOVERED
501	10^7	76.3	75.2	98.6	1.1	1.4	0.0	0.0	69	95.8	93.9	98.0	1.9	2.0	0.0	0.0
504	1.02×10^7	86.8	82.8	95.4	4.0	4.6	0.0	0.0	47	99.0	94.9	95.9	4.0	4.1	0.0	0.0
502	10^7§	51.1	46.1	90.1	0.6	0.9	4.5*	9.0*	70	69.5	63.0	90.6	0.9	1.3	5.7*‖	8.2*‖
505	1.25×10^7	60.8	54.7	89.9	6.1	10.1	0.0	0.0	72	98.2	91.2	92.9	7.0	7.1	0.0	0.0
503	10^7	36.8	19.0	51.6	2.0	5.5	15.8†	42.9†	37	47.5	26.1	54.9	5.2	10.9	15.8†	33.3†
															0.5‡	1.0‡
506	10^7	63.6	60.3	94.8	1.6	2.5	1.0*	1.6*	32	64.0	60.6	95.0	1.6	2.4	1.1*	1.7*
							0.7‡	1.1‡							0.7‡	1.1‡

*Vomitus.

†Paracenteses.

‡Sputum.

§Questionable since based upon uncertain injected dosage in Subject 502. However, the per cent of injected dosage $\times 10^5$ = CPM excreted.

‖Sweat in Subject 502 collected and measured on one day of excessive sweating yielded 15,160 CPM or 6.5 per cent of excretion of that day. There were two days of excessive sweating which would have yielded ≈ 30,000 CPM, i.e., 0.4 per cent of the total excreted or 0.3 per cent of the injected dose.

2. *Recovery of the Isotope by Various Routes.*—The duration of study of the subjects varied because the counts in the urine and serum approached background at different times and because two subjects (Subjects 503 and 506) expired at the end of the thirty-seventh and thirty-second days of observation, respectively. Therefore, the first thirty-day period of study was selected for comparison of the recovery of Cl^{36} for all patients (Table II and Fig. 4).

Fig. 4, *a* shows as a percentage of the injected dose the Cl^{36} which had been recovered by the end of the thirtieth day. A total of 76.3 and 86.8 per cent

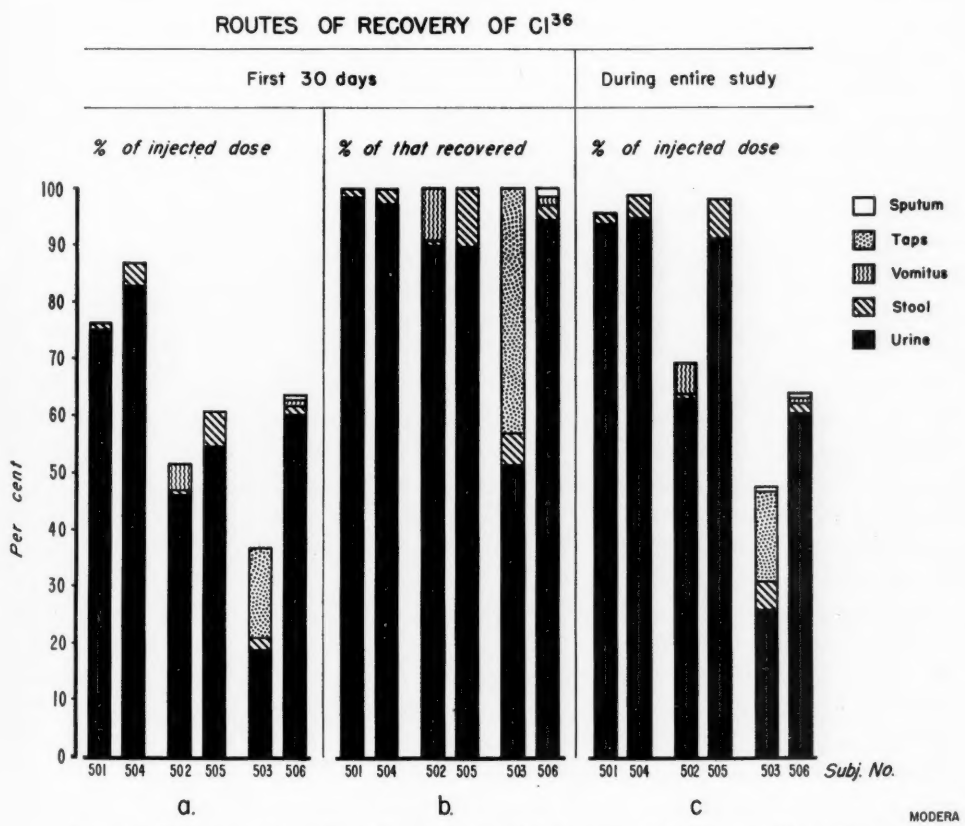

Fig. 4.—Histogram showing the routes of the recovery of Cl^{36}. *a*, Percentage of the injected dose recovered during the first thirty days of the study. *b*, Recovery of Cl^{36} expressed as a percentage of the total amount recovered in the first thirty days of the study. *c*, Percentage of the injected dose of Cl^{36} recovered during the entire period of the study. Subjects 503 and 506 expired during the course of these observations and were not observed for as long a period as the other subjects.

had been recovered in the two control subjects; 75.2 and 82.8 per cent in the urine; and 1.1 and 4.0 per cent in the stool. A total of 60.8, 36.8, and 63.7 per cent was recovered for the three patients with congestive heart failure for whom the injected dose was known: 54.7, 19.0, and 60.3 per cent in the urine; and 6.1, 2.0, and 1.6 per cent in the stool, respectively. In Subject 503, 15.8 per cent was recovered by thoracic and abdominal paracenteses, and 1.7 per cent in vomitus and sputum of Subject 506. A large portion of the urinary recovery

was obtained from a massive diuresis during the last eight days of life of Subject 506. This was responsible for a rapid decrease in serum concentration and calculated space and mass. Until the onset of this diuresis the excretory pattern of Subject 506 was similar to that of Subject 503 from whom less than 20 per cent was recovered in the urine in thirty days. The percentage of the total recovery by various routes by the end of thirty days is shown in Fig. 4, *b*.

The percentage of the administered dose recovered during the entire period of study of each patient is also presented in Table II and Fig. 4, *c*. For all subjects, except the two who expired, this time of study represents the approximate time required to reach background levels in serum and urine. For the two control subjects (Subjects 501 and 504) 95.8 and 99.0 per cent, respectively, was recovered, and 98.2 per cent was recovered for Subject 505 with congestive failure. The rate of urinary excretion in the control subjects was more rapid than in the subjects with congestive failure, but as was shown for Na^{22} during a period of rapid compensation, a patient in failure may have a more rapid excretory rate than the control subjects.[11]

Comment: Fig. 4 indicates the over-all rate of recovery for long periods of time, but the rate of excretion varied considerably during these periods, particularly under the influence of diet, diuretics, and other factors.[1, 11, 12] The recovery in all of the subjects was not complete, since two subjects died and only about 70 per cent of the injected dose was recovered from Subject 502. However, this subject perspired profusely during the experiments. On one day she excreted in sweat 15,160 CPM, or 6.5 per cent of the total excretion of that particular day (0.15 per cent of the injected dose). Therefore, although the total excretion exceeded 70 per cent, since the sweat was not measured regularly, it is possible only to estimate the total loss of Cl^{36} without including all excreted by this route.

The large recovery of the injected Cl^{36} from Subjects 501, 504, and 505 (95 to 99 per cent in 69, 47, and 72 days, respectively) indicates that there was relatively little danger from the long-term radiation effects of the long-life chloride, Cl^{36}. Since 95 to 99 per cent of the 50 μc injected was recovered, only approximately 0.5 to 2.5 μc was unaccounted for by the end of the experiment. Since the sweat and other routes of loss were not studied, and since all excreta and body fluids studied contained no detectable radioactivity, by the end of the experiments the amount remaining in the body was even less than this. As calculated from the equation* of Marinelli, Quimby, and Hine,[13] the maximal total radiation to the subject would be 0.914 e.r. for Cl^{36} with a maximal beta energy of 0.7 mev., physical half-life of 4.4×10^5 years, biological half-life of approximately 15 days and a total administered dose of 50 μc.

In subjects with the biologic half-life periods longer than 15 days due to low rates of excretion such as in association with low chloride intake and chronic

*$D_b = K_b C$ e. r.,
D_b = total dose in equivalent roentgens,
C = concentration in μc per gram of tissue,
K_b = 88 $E_b T$ e. r. per μc destroyed per gram,
E_b = average beta energy in mev. and
T = effective half-life (in the case of Cl^{36}, the biologic half-life in days),
C = 50/50,000 for a 50 Kg. patient = 1^{-3} μc per gram,
K_b = $88 \times 0.7 \times 15 = 914$ e. r. per μc per gram, and
$D_b = 914 \times 10^{-3} = 0.914$ e. r.

congestive heart failure, the total maximal body irradiation will be greater than 0.914 e.r. Conversely, a high chloride intake or procedures which hasten the excretion of chloride would reduce the total maximal radiation. The implications of this are apparent.

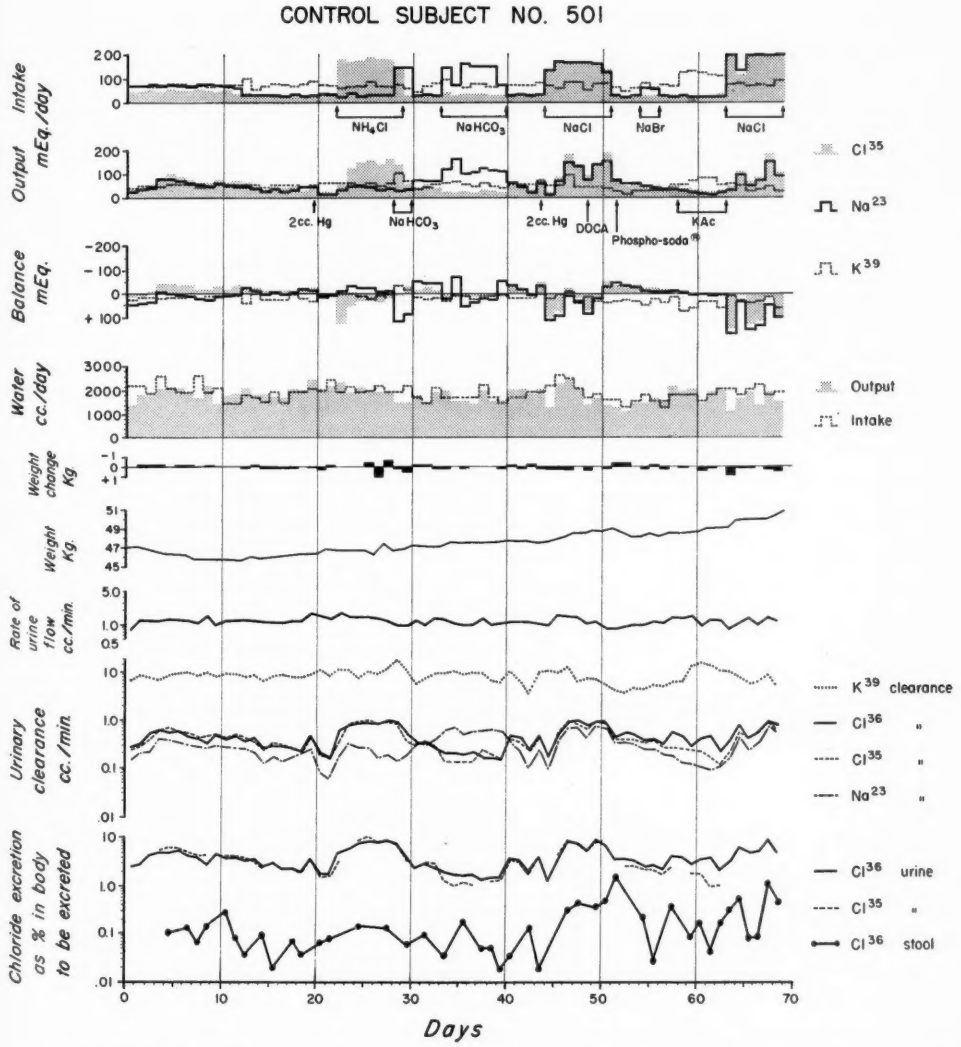

Fig. 5.—The simultaneous time-course curves of various parameters obtained on control Subject 501, including balance data. The time and duration of administration of various drugs as well as other special procedures are indicated.

These experiments of recovery indicate that very little, if any, Cl^{36} was "permanently" firmly bound in noncommunicating stores. If such stores existed, however, the Cl^{36} could have been "functionally sequestrated" by slow rates of exchange.[2, 3]

3. *Daily Excretion, Clearance, and Balance of Cl^{36}, Cl^{35}, Sodium, and Potassium.*—

Data concerned with the daily excretion of chloride, sodium, potassium, and water are summarized in Figs. 5 through 10 to show the relationships among various factors which may have influenced excretion. In each figure are shown the daily average rate of urine flow in milliliters per minute; the average daily

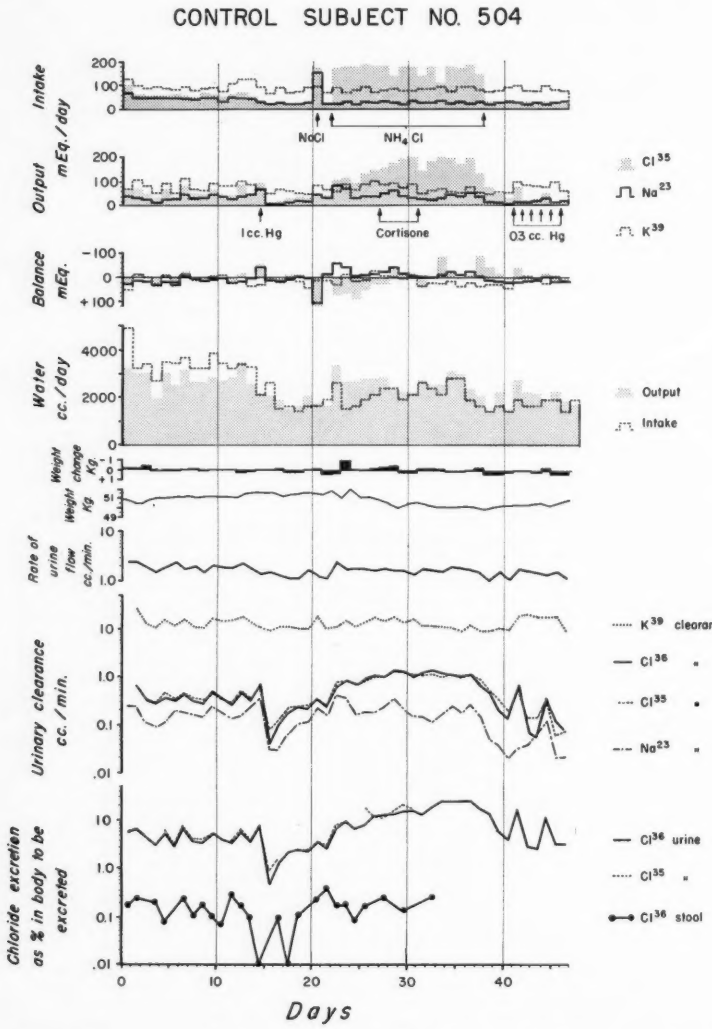

Fig. 6.—The simultaneous time-course curves of various parameters obtained on Subject 502, including balance data. The time and duration of administration of various drugs as well as other special procedures are indicated.

clearance of radiochloride, chloride, sodium, and potassium; the total daily urinary excretion of radiochloride and chloride and the daily fecal excretion of radiochloride as a percentage of that present in the body; the daily intake, output and balance of chloride, sodium, potassium, and water; and the body weight and the change in body weight in kilograms. Drugs and procedures employed during the study are also indicated.

The daily excretion of radiochloride is expressed as a percentage of that remaining in the body. This unit was employed to facilitate interpretation of the excretory phenomena at the later periods in the study when the counts approached background and actual counts expressed as a percentage of the injected dose would have produced a distorted picture of excretory function. This also permits comparison of the excretion of Cl^{36} with that of Cl^{35} expressed as a percentage of the *calculated* total amount of Cl^{35} in the body. These calculations were obtained in the following manner:

$\frac{N_{Kt}}{N_T} \times 100$ = excretion on day t as a percentage of the total Cl^{36} remaining in the body

where N_{Kt} = measured amount of Cl^{36} excreted in CPM on the t^{th} day, and

N_T = amount of Cl^{36} remaining in the body at the beginning of the t^{th} day in CPM or $N_o - \sum_{s=1}^{t} k_s$,

where N_o = the injected dose in CPM.[11]

The excretion of Cl^{35} on any day t expressed as a percentage of the *calculated* mass of chloride in the body was obtained by the equation:

$\frac{K_t}{M_T} \times 100$ = excretion of Cl^{35} as a percentage of calculated chloride mass,

where K_t = measured amount of Cl^{35} excreted in grams per day and
M_T = calculated mass of chloride in the body in grams.

These calculations may be applied to any route or routes of excretion, such as urine, stool, vomitus, sputum, or other normal or abnormal biologic fluids.

The general variations in the parameters for each subject were similar (Figs. 5 through 10). For example, when there was an increase in the magnitude of urine flow, there was an increase in clearance rates, and percentage excretion of the substances measured, as well as a total quantitative increase in these substances. With exceptions which will be discussed in detail later, the variations conformed with those expected from the changes in weight and electrolyte balances.

A. *Excretion of Cl^{36} and Cl^{35} as a percentage of that present in the body:* As indicated previously in studies of the biologic decay rates of Na^{22} and Cl^{36}, these rates could be made to vary by changing intake of the respective stable elements. This is exemplified in Figs. 5 and 6 for control Subjects 501 and 504, who excreted 9 and 20 per cent, respectively, while on high chloride intake (175 to 200 meq. per day) and 4 to 5 per cent during periods of low intake (25 to 40 meq. per day).

The average percentage output was maintained at a fairly high level as a result of the response to ammonium chloride, mercurial diuretics, and general improvement in Subject 505 (Fig. 8), who was edematous initially but improved rapidly throughout the study. There were wide variations in these values which reached 10 to 11 per cent during the period of rapid excretion and even 26 per

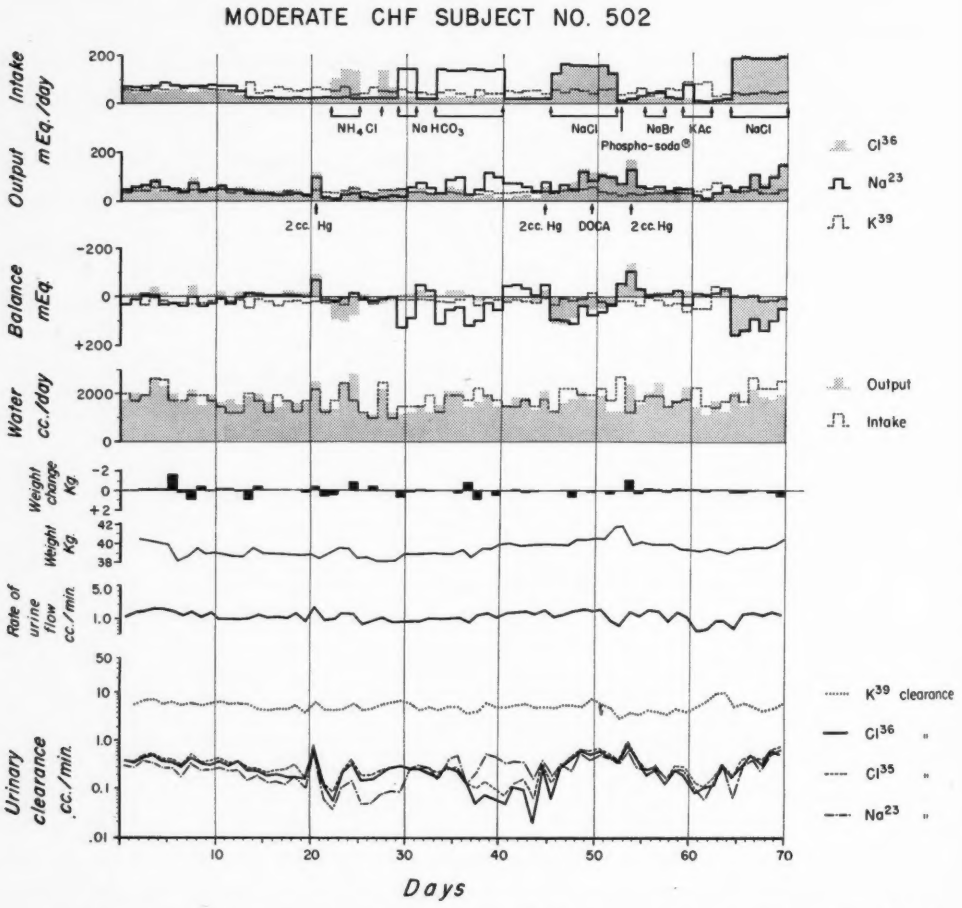

Fig. 7.—The simultaneous time-course curves of various parameters obtained on Subject 503, including balance data. The time and duration of administration of various drugs as well as other special procedures are indicated.

cent after administration of a mercurial diuretic. The percentage excreted was extremely low, less than 1 per cent for the two subjects with severe failure (Figs. 9, 10), except for days when mercurial diuretics were given in Subject 506 (Fig. 10) for the ten days preceding her death when increased excretion was maintained with ammonium chloride, aminophylline, and mercurial diuretics. The day immediately prior to death, the percentage excretion returned to less than 1 per cent. The relationship of the variations in the excretion of Cl^{35} and Cl^{36} as a percentage of that in the body to be excreted to the variations

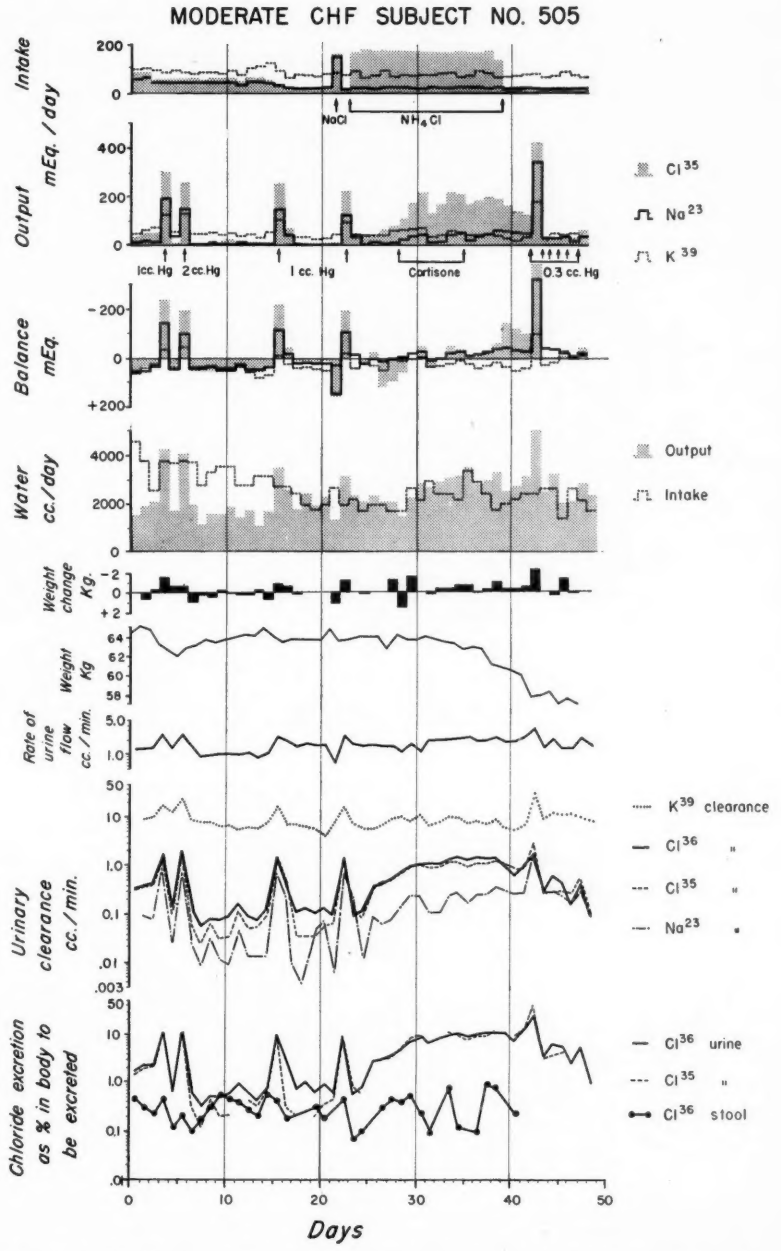

Fig. 8.—The simultaneous time-course curves of various parameters obtained on control Subject 504, including balance data. The time and duration of administration of various drugs as well as other special procedures are indicated.

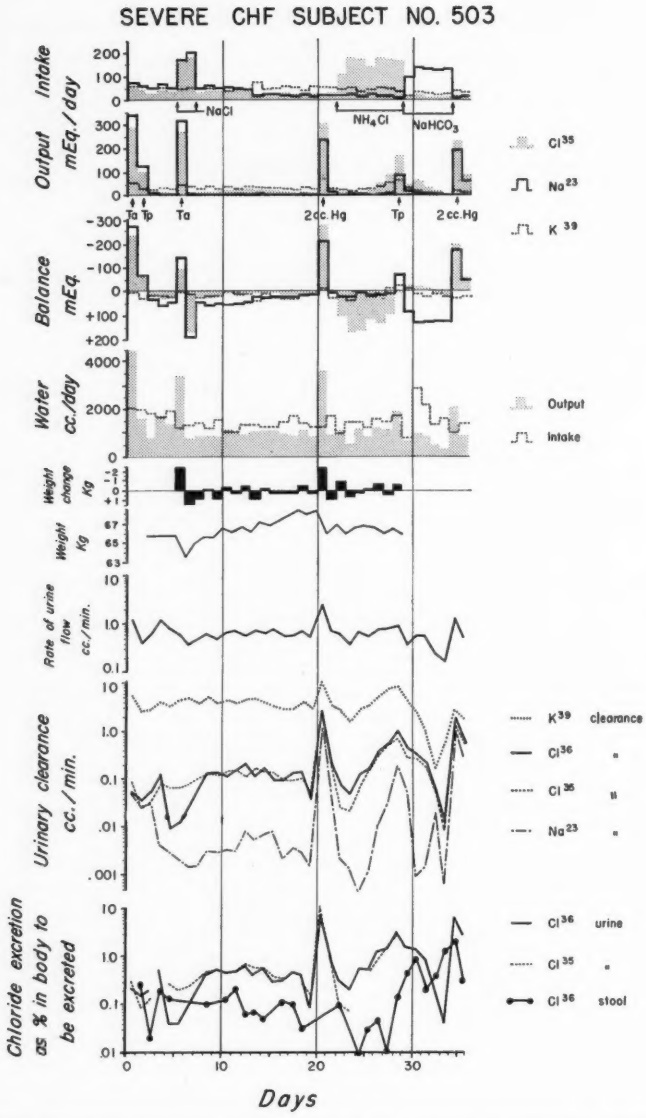

Fig. 9.—The simultaneous time-course curves of various parameters obtained on Subject 505, including balance data. The time and duration of administration of various drugs as well as other special procedures are indicated.

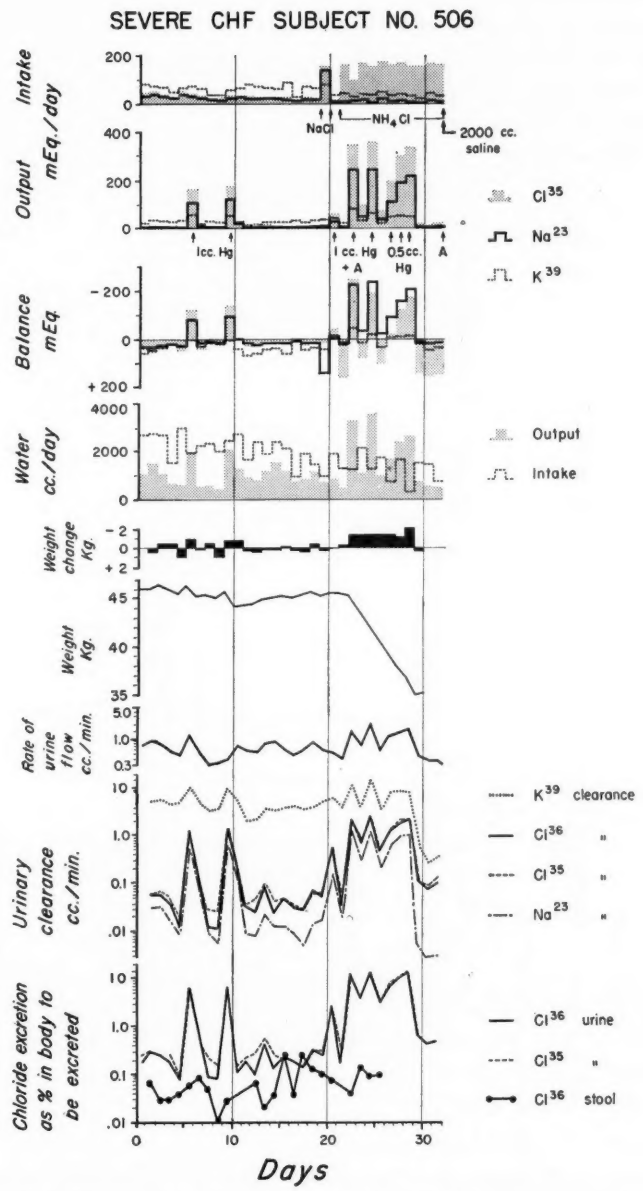

Fig. 10.—The simultaneous time-course curves of various parameters obtained on Subject 506, including balance data. The time and duration of administration of various drugs as well as other special procedures are indicated.

in the excretion of sodium, potassium, and water, as well as the other time-course curves shown in these figures, can be obtained only by careful study of the illustrations.

Selected aspects of the time-course curves of Cl^{36} excretion expressed as a percentage of that remaining in the body are summarized in Fig. 11 and Table III. The values presented are means of several days of observation during which the effect of certain procedures and drugs were tested. The illustration indicates the following:

TABLE III.

SUBJECT	AVERAGE DAILY EXCRETION ON LOW DIET (PER CENT PRESENT)	AVERAGE DAILY EXCRETION ON Hg AND LOW DIET (PER CENT PRESENT)	AVERAGE DAILY EXCRETION ON DAY AFTER HG (PER CENT PRESENT)	RATIO Hg DAY: CONTROL DAY	RATIO DAY AFTER Hg: CONTROL DAY	RATIO AV. DAILY EXCRETION OF Hg DAY AND DAY AFTER: CONTROL DAY
501 (Control)	3.55	4.11	1.65	1.16	0.46	0.81
504 (Control)	3.91	5.58	0.47	1.43	0.12	0.78
505 (Mod. CHF)	0.66	10.45	1.26	15.83	1.91	8.87
503 (Sev. CHF)	0.38	7.47	1.80	19.66	4.74	12.20
506 (Sev. CHF)	0.23	6.65	0.79	28.91	3.43	16.17

CHF = Congestive heart failure.

1. On both high (175 to 200 meq. per day) and low (25 to 40 meq. per day) chloride diets, the nonedematous control subjects excreted a greater percentage of the Cl^{36} remaining in the body than did the subjects with congestive heart failure. Subject 505, who compensated and who has remained essentially in the compensated state for three years since completion of these experiments, was able to excrete chloride at a considerably greater rate when on a high chloride diet than those subjects in severe failure (Subjects 503 and 506).

2. The patients with congestive heart failure excreted more Cl^{36} in response to the mercurial diuretics than the control nonedematous subjects. When the dietary intake of chloride was low, the excretion of Cl^{36} by the control subjects averaged 1.16 and 1.43 times that on the control days, whereas the subjects with congestive heart failure excreted an average of 15.83 to 28.54 times more than on a control day (Table III).

3. The control subjects showed a greater tendency to retain Cl^{36} on the day following a mercurial diuresis than the subjects with congestive heart failure (Table III). On the day of mercurial diuresis the control subjects excreted from 20 to 40 per cent more Cl^{36}, and 90 to 50 per cent less Cl^{36} on the day after diuresis than during the control period preceding the administration of the mercurial diuretic. The net output for these two days was approximately 20 per cent less than for the control period. Subjects with congestive heart failure responded with a sixteen- to thirty-fold increase in

excretion of Cl^{36} on the day of administration of mercurial diuretics and on the next day the excretion was two- to five-fold greater than the control with the net excretion for the two-day period from nine to seventeen times the control level.

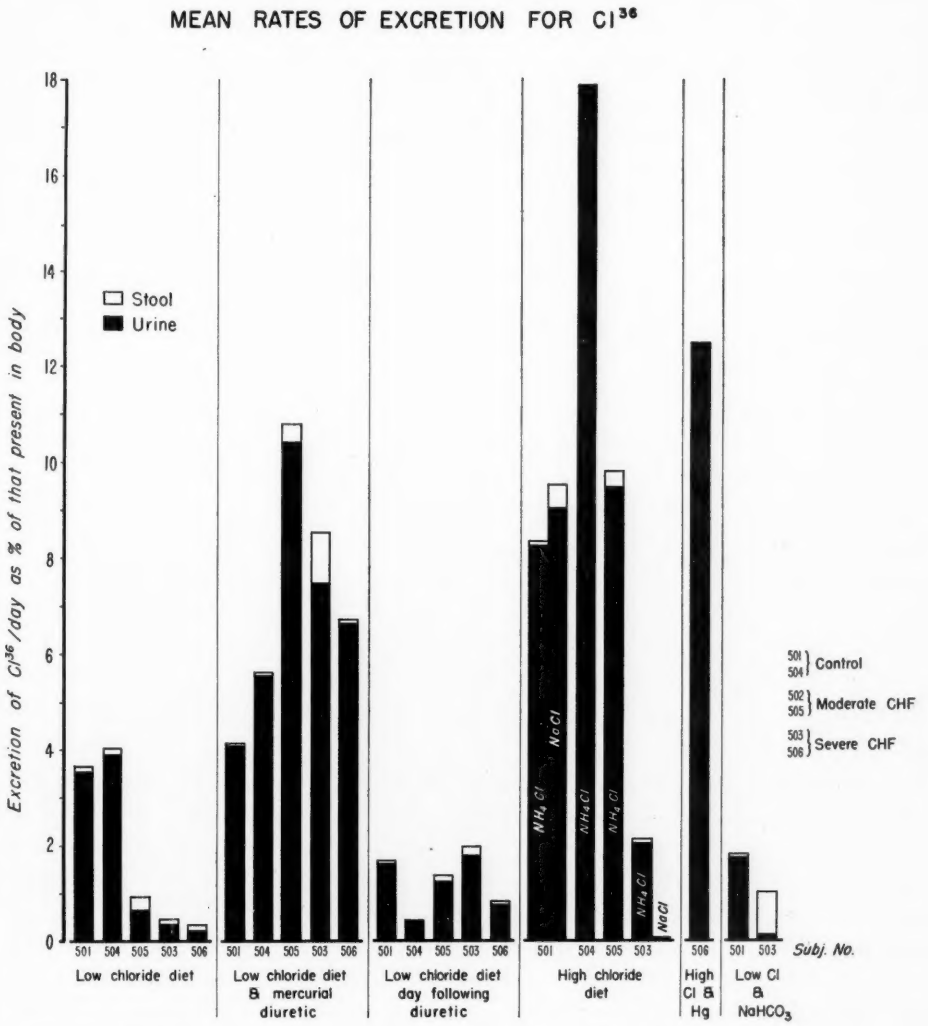

Fig. 11.—Histogram showing the effects of dietary intake of Cl^{35}, mercurial diuretics, and sodium bicarbonate upon the averages of the rates of excretion of Cl^{36} expressed as percentage of the Cl^{36} remaining in the body to be excreted.

4. One control subject and one subject with congestive heart failure who received sodium bicarbonate manifested definite reduction in the excretion of chloride associated with an increase in the calculated chloride space. This finding had been previously interpreted[2, 3] to indicate a shift of chloride into the intracellular compartment, an observation corroborated by Pitts and Axelrod.[14]

5. The percentage of Cl^{36} excreted in the stool was relatively small, varying between less than 1 and 9 per cent. The highest values were observed in the patients with congestive heart failure during periods of low urinary excretion of chloride.

Subject 501 (Fig. 5) while on sodium chloride manifested an increase in the excretion of Cl^{36} in stools. When on ammonium chloride, however, there was no such increase in the fecal excretion of the isotope for either control Subject 501 (Fig. 5) or Subject 504 (Fig. 6) for whom the excretion of radiochloride in the stool was remarkably constant. The patients with congestive heart failure, on the other hand, tended to show a greater variation in the percentage fecal excretion, which often exceeded excretion in the urine as did loss by paracenteses.

Comment: The time-course curves of Cl^{36} and Cl^{35} excretion as a percentage of that remaining in the body showed an expected similarity. Nevertheless, there were notable discrepancies, the precise cause for which is unknown. These discrepancies may be due to errors in measurement of the stable and tracer isotopes, errors in the calculation of Cl^{36} and Cl^{35} remaining in the body due to failure to collect all losses, inadequacies of equilibrium of distribution of the isotope or ingested Cl^{35}, sequestration of tracer and/or nontracer, as well as other physiologic phenomena related to disturbances in equilibrium of distribution of the tracer and the steady state of chloride metabolism.[3] The failure to maintain proper temporal relationship among the various measurements employed in the calculation of these curves may be another cause for the discrepancies. For example, if there was change in the concentration of tracer or nontracer in the serum during the course of a day, calculations based on measurements made at different times of the day would result in the discrepancies noted in these curves.

When the previously mentioned difficulties are considered,[3] the two control subjects showed remarkable similarity of the time-course curves of Cl^{35} and Cl^{36} expressed as percentage of that remaining in the body for excretion. The subjects with congestive heart failure, in contrast, showed considerable fluctuations in the magnitude of these time-course curves and in the discrepancies in values between them. If the system were homogeneous, the physiologic state steady, and the equilibrium of distribution of the tracer fully established throughout all chloride compartments of the body, these two curves would be superimposed. Therefore, these excretory curves indicate further that the patients with congestive heart failure were unable to maintain a stable physiologic state of extracellular fluid, whereas the control subjects were able to do so. This confirms further conclusions derived from the study of space and mass of Cl^{36} reported previously.[2, 3]

As indicated previously,[3, 12] the excretion of tracer would be expected to vary inversely with the space of the nontracer if the diet remained constant. To investigate this concept further, the percentage of the tracer excreted was plotted against the calculated mass of Cl^{35} percentage of the body weight (Fig.

12). As was expected they were inversely related; and, furthermore, the same inverse relationship was found to exist between excretion of Cl^{36} and mass of Cl^{35} expressed in grams or as a percentage of the body weight.

B. *Urinary clearance of Cl^{36}, Cl^{35}, sodium, and potassium:* The urinary clearance rates for all the substances studied, including water, were highest for the two control subjects and the subjects with moderate congestive heart failure who were compensating; they were lowest for the subjects with severe congestive heart failure (Figs. 5 through 10). Increase in the intake of any of the substances resulted in an associated increase in the rate of clearance of that substance. Those patients with severe congestive heart failure, however, did not always respond as well as the control subjects. Mercurial diuretics produced large increases in the clearance rates, especially in the subjects with congestive heart failure.

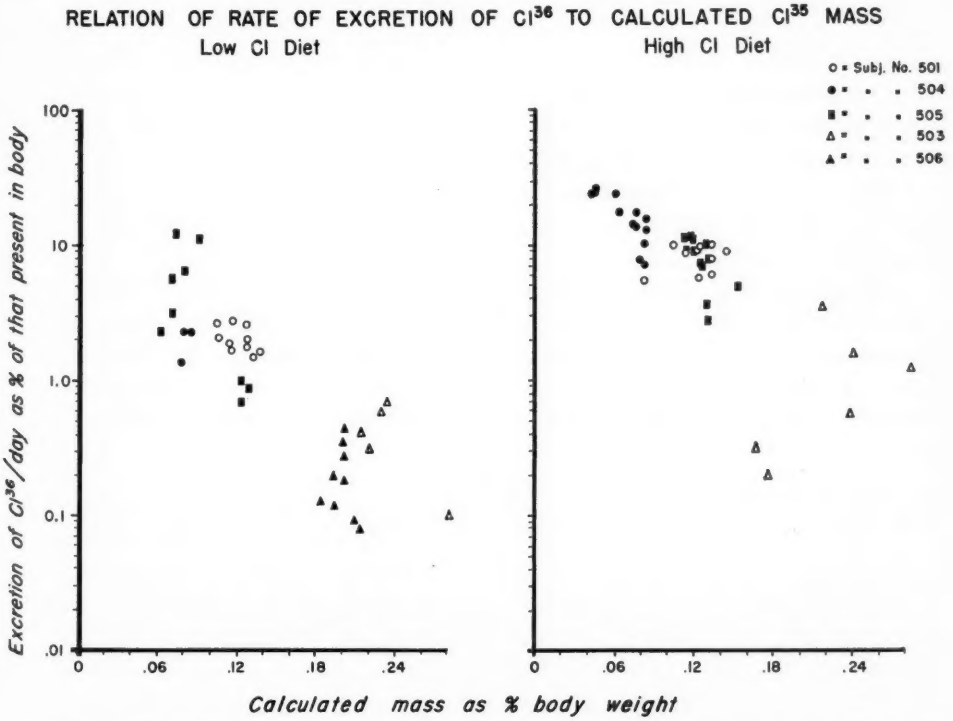

Fig. 12.—Scatter graph of the rate of excretion of Cl^{36} expressed as percentage of tracer present in the body to be excreted related to the calculated mass of Cl^{35} expressed as percentage of body weight for five subjects on a low and a high chloride intake.

Although there was a tendency for concordant variations in the curves of values of sodium, potassium, and chloride clearance, there were times when these diverged (Figs. 5 through 10). The clearance of potassium in control Subject 504 (Fig. 6) was approximately thirty-five times the chloride clearance on low chloride diet and twelve to fifteen times that on a high chloride diet, the range being 8 and 474. The chloride clearance was approximately three times the sodium clearance. Sodium clearance never exceeded chloride clearance in

Subject 504 (Fig. 6) and Subject 506 (Fig. 10) whose dietary intake of sodium never exceeded that of chloride. The sodium clearance exceeded the chloride clearance in Subject 501 (Fig. 5) and Subject 502 (Fig. 7) only when they received sodium bicarbonate. In spite of an intake of sodium exceeding that of chloride during the administration of sodium bicarbonate, Subject 503 (Fig. 9) never had a sodium clearance higher than chloride clearance.

Comment: The clearance of Cl^{36} was quantitatively similar to that of Cl^{35}. The differences noted may have been due to the factors discussed for differences in percentage excretion. Chloride clearance and sodium clearance tended to vary concordantly except when one or the other element was varied independently in the diet. Potassium clearance, on the other hand, tended to vary relatively little during these experiments and did not necessarily vary concordantly with chloride and sodium clearances. The independence of the potassium clearance in these experiments may be related in part to the relatively high potassium intake and because the greater mass of potassium is intracellularly located and the serum concentration is low. Because of the high intake and the low serum concentration, the clearance of potassium was relatively high with respect to sodium and chloride. The high potassium clearance indicated a rapid turnover of potassium in the blood serum.

The control subjects responded promptly to changes in the dietary intake of these elements whereas the subjects with congestive heart failure did not necessarily do so.

Because of the numerous variations in the clearance curves of the different elements (Figs. 5 through 10), it is difficult to determine by mere inspection of the curves whether or not the clearance rates vary because of differences in dietary intake and/or serum concentration. It is possible, therefore, to make corrections for dietary intake and serum concentration by the following equation:

$$\frac{\text{Av. daily Na clearance} \times \frac{\text{Serum Na}}{\text{Serum Cl}} \times \frac{\text{Cl Intake}}{\text{Na Intake}}}{\text{Av. daily Cl clearance}} \equiv \frac{\text{Na output/day}}{\text{Cl output/day}} \times \frac{\text{Cl intake/day}}{\text{Na intake/day}} =$$

corrected sodium clearance as a fraction of chloride clearance. When this ratio is applied to the Cl^{36}, Cl^{35}, sodium, and potassium clearance curves of Subject 501 (Fig. 5), and plotted (Fig. 13), it is possible to determine readily by inspection whenever a disproportional clearance change develops. Because of the nature of this ratio, the direction and absolute amounts of change cannot be determined from the ratio alone. By referring to Fig. 5 for the appropriate time, however, it is possible to determine these quantitative changes responsible for the changes in the ratio. For example, in Fig. 13 definite changes occurred in the ratio at the time of administration of ammonium chloride, sodium bicarbonate, sodium chloride, and potassium acetate. The direction and quantities responsible for these changes are available from the balance and the clearance curves shown in Fig. 5. No attempt was made to present charts of such ratios for each of the subjects studied.

The clearances presented are average daily clearances derived from clearances which were measured at much shorter intervals during each day. The

moment-to-moment variations during the day were considerably greater than the daily average clearances. These marked variations indicate the possible erroneous impression which may be obtained concerning the excretion of these elements when single clearances are measured over short intervals. Serial and numerous measurements are necessary under controlled metabolic conditions to provide a more accurate pattern of the time course of clearance.

C. *Specific activity:* The time course of daily specific activity (Cl^{36}/Cl^{35}) in the blood and urine of all the subjects is summarized in Fig. 14. The specific activities of the blood and urine follow each other closely with divergence being more frequent and of greater magnitude in the subjects with congestive heart failure than in the control subjects. The time-course curves of Cl^{35} and Cl^{36} clearance are also presented in Fig. 14 to illustrate the

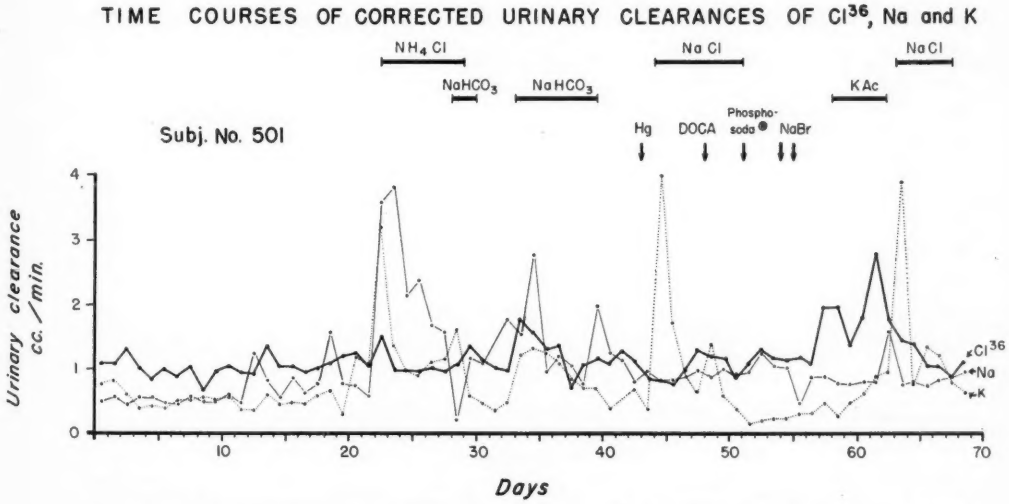

Fig. 13.—The time course of urinary clearances of Cl^{36}, sodium, and potassium in which corrections were made for differences in dietary intake and serum concentration for control Subject 501. Consult text for details.

simultaneous variations in the specific activities and clearances. It is evident that the greatest discrepancies in specific activities of urine and blood occurred when the rates of Cl^{35} and Cl^{36} clearance showed the greatest changes and discrepancies. Also, as would be expected, the rate of regression of specific activity in the blood and urine was the same as the biologic decay rates of Cl^{36} in the blood.[1] It is noted, for example, for Subject 506 with the rapid rate of clearance of Cl^{35} and Cl^{36} due to the elimination of edema fluid, that the specific activity of the blood and urine declined rapidly.

Comment: Were the physiologic system noncompartmented, equilibrium of distribution established and a steady state maintained, and measurements free from significant error, the time-course curves of specific activity of the blood and urine would be superimposed. Because such conditions did not exist, there were discrepancies between the urine and blood specific activities. The reasons for these discrepancies have been discussed earlier in this report.

The great discrepancies in specific activity in the patients with congestive heart failure is further evidence of the instability of the state of chloride metabolism in this clinical condition. This instability in specific activity in the blood as well as in the urine indicates instability of intracellular in addition to extracellular chloride metabolism in congestive heart failure. The greatest discrepancies in specific activities of urine and blood were noted (Fig. 14) during periods of large fluctuation in chloride metabolism as occurred in association with variations in the state of congestive heart failure, administration of mercurial diuretics, and administration of chloride.

D. *The interrelationship of balance of chloride to balance of sodium, potassium, and water:* The time courses of chloride, sodium, potassium, and water balance are summarized in Figs. 5 through 10. Inspection of these curves reveals the interrelationship of these balance measurements with such factors as body weight, clearance of chloride, sodium, potassium, Cl^{36}, the use of drugs or other special procedures, and variations in intake of electrolytes and water. All of the subjects were on a relatively low chloride intake ($\approx$ 30 meq. per day) for about seven days before initiation of these experiments, and throughout the experiments except when indicated on the illustrations.

After being placed on a greater intake of chloride, the control subjects retained chloride in large quantities for the first two days and then gradually came into balance in about six days (Figs. 5 and 6). The subjects with mild congestive heart failure, those who were recovering from their failure, had a response which was similar to the control subjects. On the other hand, Subject 503 with severe congestive heart failure tended to remain in positive chloride balance. Subject 506, who was receiving aminophylline and mercurial diuretics simultaneously with the increased intake of chloride as ammonium chloride, had a negative balance attributable to the effects of the drugs. Ammonium chloride increased sodium excretion in control Subjects 501 and 504 and in Subject 505 with mild congestive heart failure. The excretion of potassium was not measurably altered by ammonium chloride in any of the subjects.

Subjects 501 and 502, who had adequate periods of increased oral intake of sodium chloride, retained both sodium and chloride. The retention of chloride was greater when control Subject 501 was on sodium chloride than when chloride was administered as ammonium chloride. In none of the subjects was excretion of potassium altered by the administration of sodium chloride under these conditions of administration. A negative balance, a "rebound" phenomenon, was present at the end of the periods of high intake. There was no increase in body weight in association with a positive chloride balance due to high intake of ammonium chloride, but there was a gain in body weight in association with the positive chloride balance due to an increased intake of sodium chloride.

Sodium and chloride balances were negative while potassium remained unchanged during the excretion of edema fluid by the patients recovering from heart failure. Sodium, chloride, and potassium balance varied slightly during the periods of constant dietary intake and essentially a constant physiologic

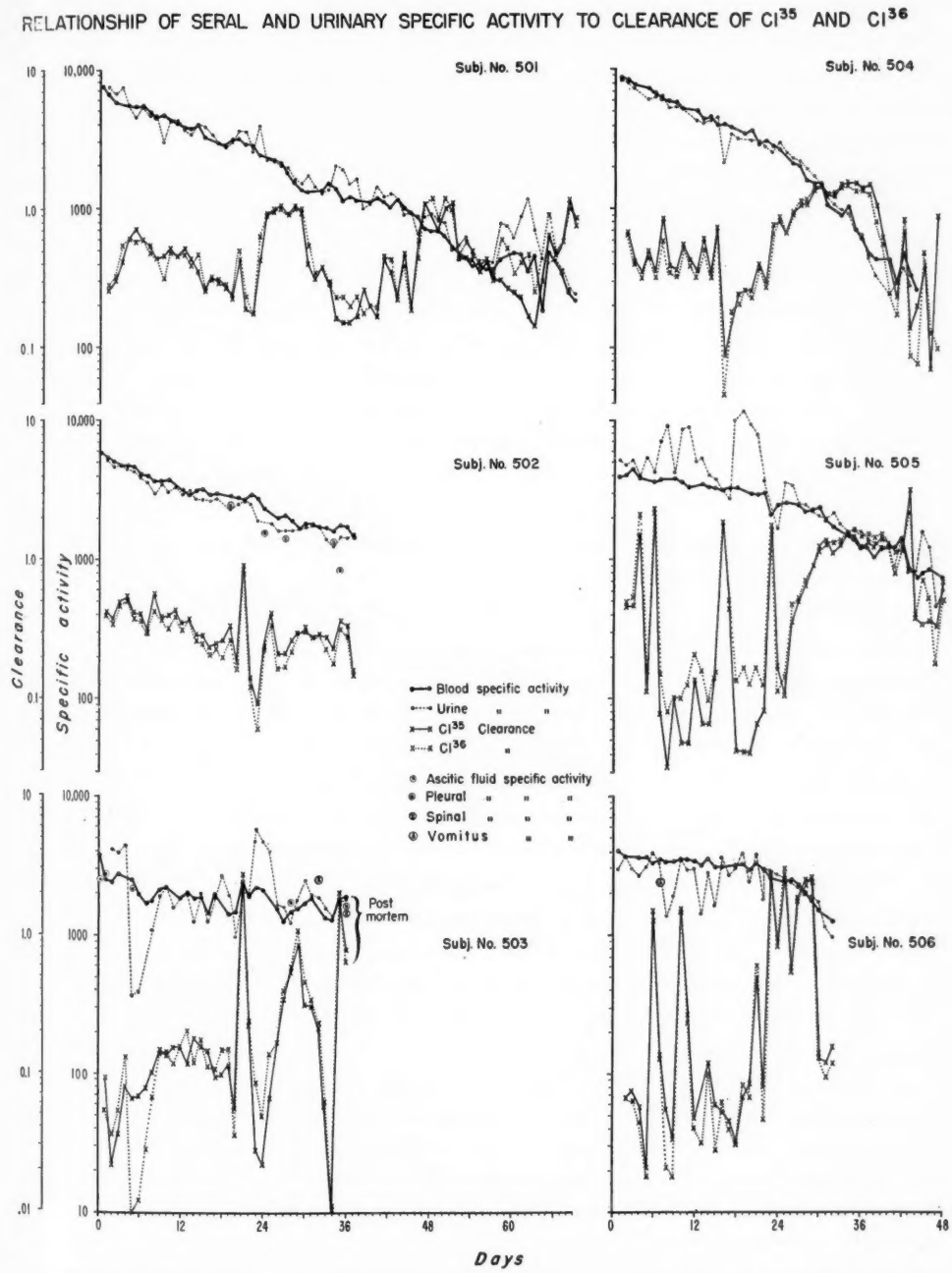

Fig. 14.—Simultaneous time-course curves of serum and urine specific activity and Cl^{35} and Cl^{36} clearances for the six subjects studied. The specific activities of other isolated biologic fluids are plotted as separate points. A careful study of this illustration reveals many interesting relationships among these curves.

state (Figs. 5 through 10). Sodium and chloride balances varied considerably with increases in output of these electrolytes produced by the administration of mercurial diuretics, whereas the potassium balance remained essentially constant.

Comment: Because these subjects were on low sodium and chloride intakes, it is not surprising that following the administration of sodium and chloride there was a tendency to retain these elements for several days with a gradual approach to a zero balance value. When the dietary intakes of these elements were again reduced, there was a rebound negativity in the balance state due to a lag in the reduction of the excretory rate. The negative rebound in sodium or chloride balance following the reduction in the administration of one or the other may be an expression of the normal physiologic "hunting" phenomenon, that is, the physiologic state being maintained at some constant level about which there is slight undershooting or overshooting. With the sudden withdrawal of an electrolyte the mechanisms associated with reduction in output of the electrolyte probably have not become fully developed to prevent negative balance or excessive excretion. This is especially noticeable in subjects with tuberculosis.[15] The patient with progressing and severe congestive heart failure failed to show the same excretory responses to increases in chloride and sodium intake manifested by the control subjects.

The discrepancy between chloride and sodium balance produced by the administration of one or the other electrolyte alone demonstrated that the measurement of either sodium or chloride balance or the excretion of either electrolyte in the urine will not always indicate the state of balance or rate of excretion of the other element. Therefore, claims that the study of chloride in the urine can serve as an index of urinary excretion of sodium are not substantiated as being reliable under all circumstances, particularly under the influence of drugs and variation in the physiologic state.

It is usually customary for investigators to report the data of metabolic studies concerned with balance measurements for any substance as the net *daily* balance. When the net cumulative balance is recorded for many consecutive days of observation, such as in these experiments, it becomes evident that the net balance obtained for prolonged periods results in large net values. Inspection of Fig. 15, showing the time course of the cumulative net balance of sodium, potassium, and chloride reveals, in general, a constant gain in potassium for all of the subjects which was out of proportion to that which was expected for the associated change in body weight. Although not shown, the same phenomenon was true for water. Obviously, this was physiologically impossible and must have been due to the net effects of slight and constant errors in measurement. For example, the failure to measure accurately potassium intake and potassium excretion in the stool and sweat, and insensible water loss was most likely responsible for the constant net positive balances of potassium and water and the resultant large positive balances at the termination of the studies. This may also be in part due to errors in the potassium values in the food tables.[4] The type of "bookkeeping" illustrated by Fig. 15 can be very useful in the revelation of small errors in technique.

It should be noted that for Subject 503 on some days a large negative balance was produced by the quantity of electrolytes removed by paracenteses without any significant change in urinary excretion. The positive balance of other subjects probably represents losses in excreta other than urine.

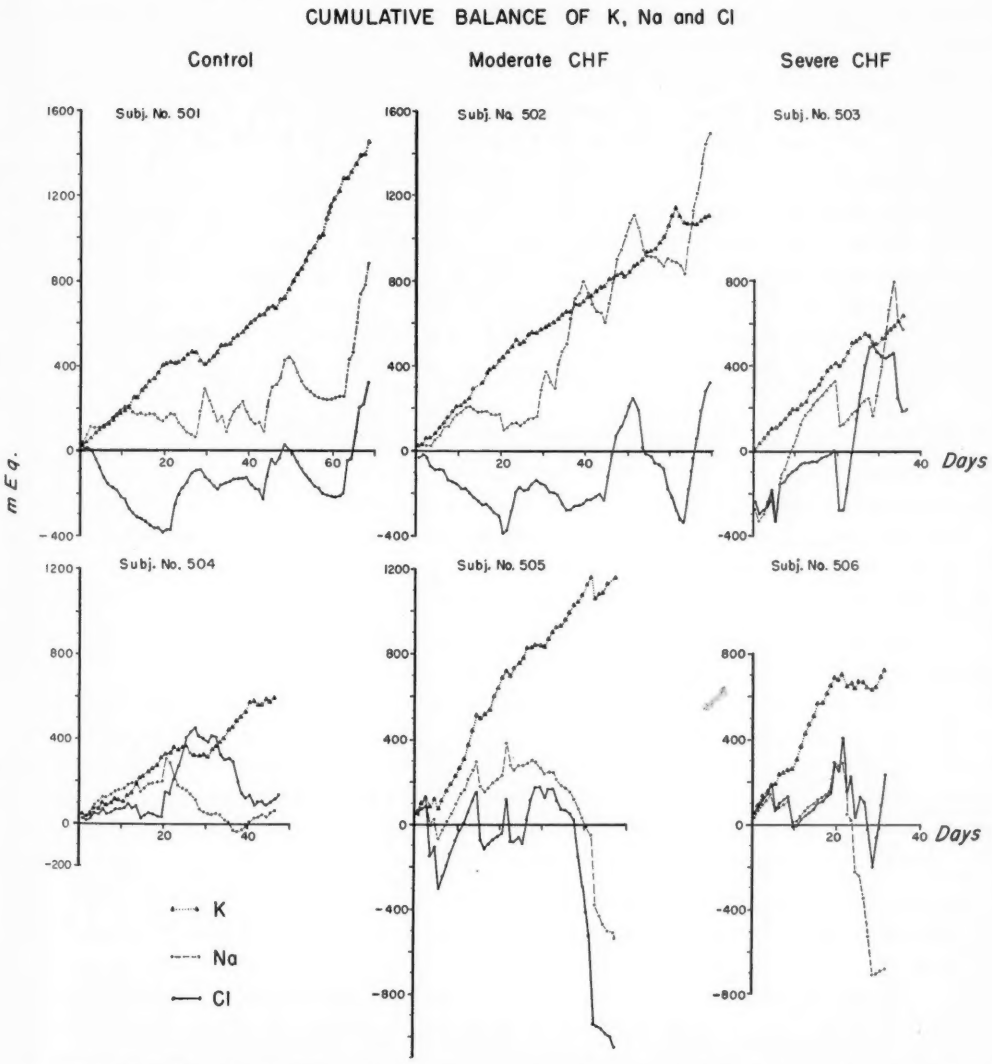

Fig. 15.—The simultaneous time-course curves of accumulated balance of chloride, sodium, and potassium for the six subjects studied, as obtained during the entire period of observation.

4. *The Influence of Drugs and Special Procedures Upon the Excretion of Chloride, Sodium, and Potassium.*—

Figs. 5 through 11 summarize in general the influence of special procedures and drugs upon the various parameters observed in these experiments. Careful inspection of these illustrations will reveal the influence of these procedures upon the various individual curves and the interrelationships of the variations among the curves. Certain aspects of these responses are considered here.

A. *Intravenous administration of sodium chloride:* Two hundred and fifty milliliters of 3 per cent sodium chloride (7.5 Gm. or 128 meq.) in water was administered intravenously to three of the subjects, 504, 505, and 506, over a period of one hour. Catheters had been inserted into the bladder prior to the infusion of the saline and urine samples were collected at fifteen-minute intervals. Samples of blood were collected at thirty-minute intervals. These collections were continued for two hours; then each specimen of urine was collected as voided in a separate container. From the data obtained, time-course curves of Cl^{36}, Cl^{35}, sodium, and potassium concentration in the blood serum and urine, clearance for each of these, specific activity in the urine and serum, and rate of urine flow were plotted (Fig. 16). The subjects had had no special preparation prior to the infusion, having been on water *ad libitum* and a relatively low sodium and chloride intake for about three weeks preceding the infusion. Excretion of water declined precipitously in the normal subject and the subject with severe congestive heart failure and to a lesser degree in the subject with moderately severe congestive heart failure.

The antidiuretic effect of the hypertonic solution in two of these subjects was similar to that described for the dog in the studies of Verney.[16] Excretion of potassium was little affected by the infusion. The Cl^{36}, Cl^{35}, and sodium clearances were much higher in the control subject than in the two with heart failure (Fig. 16). These findings further demonstrate the impairment in congestive heart failure of the physiologic functions responsible for the maintenance of the steady state of electrolyte constitution of body fluids.

B. *Mercurial diuretics:* Catheters were placed in the urinary bladder and one milliliter of a mercurial diuretic (Mercuhydrin) was administered intravenously to Subjects 504 and 505. Samples of urine were collected at thirty-minute intervals for two and one-half hours, and then each voided specimen was collected in separate containers as passed. Samples of blood were taken at the beginning of the study one hour before the administration of the diuretic. From these data time-course curves were plotted (Fig. 17) of the concentration of Cl^{36}, Cl^{35}, sodium, and potassium in the urine, the clearances of each of these elements, specific activity of each specimen of urine and of blood serum for the morning of the injection and thirty-six hours later, and the rates of urine flow.

There was definite diuresis (Fig. 17) in both the control subject (Subject 504) and the subject with congestive heart failure (Subject 505). This diuresis was sustained for a longer period of time in the patient with congestive heart failure (Fig. 17). The control subject showed a "rebound" antidiuretic response during the latter part of the first twenty-four hours following the administration of the diuretic (Figs. 17 and 11). The longer period of diuresis in the subject with congestive heart failure is possibly related to the slower excretion of the mercury observed in other studies of the excretion of a diuretic labelled with radiomercury.[17]

The diuresis produced by the mercurial diuretic was manifested by an increase in the concentration of Cl^{36}, Cl^{35}, and sodium. The increase in concen-

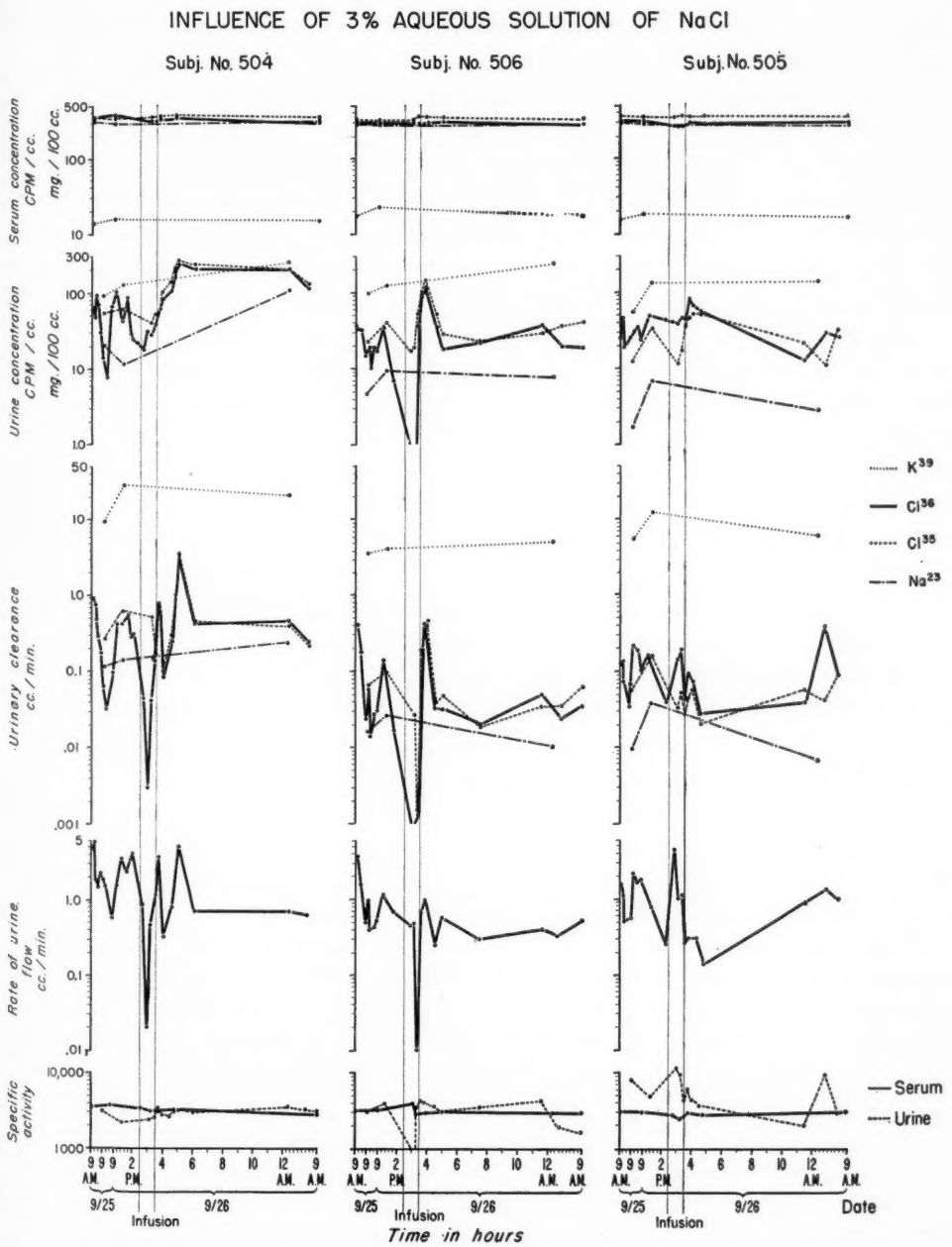

Fig. 16.—The influence in three subjects of the intravenous administration of 3 per cent aqueous (250 ml.) of NaCl upon the serum and urine concentration and urinary clearance of Cl^{36}, Cl^{35}, sodium, and potassium as well as upon the rate of urine flow and the specific activity of Cl^{36} in both serum and urine.

tration of potassium in the urine was less than that of sodium and chloride. There was also an increase in the clearance rates of these elements as well as of water. There was a striking parallelism in the water and electrolyte excretion in both patients evident in Fig. 17.

The excretion of potassium was increased only slightly in the subject with moderate congestive heart failure and in the control subject. As can be seen from study of Figs. 5 through 10, the response in the excretion of potassium to a mercurial diuretic varied from no response to a moderate increase when compared with the excretion of sodium, chloride, and water.

The definite response on the part of the kidneys to the mercurial diuretic in the subject with congestive heart failure indicates the ability of the kidney in this clinical state to function well when influenced by a proper agent. This is in support of an alteration of renal function in congestive heart failure rather than renal disease with organic impairment, for were the kidneys so greatly diseased in congestive heart failure that they were unable to produce urine, they would not be expected to respond as markedly to the mercurial diuretic as observed in these experiments. In general, the degree of response to the mercurial diuretic was directly related to the severity of the failure, the control subjects showing the least diuresis, and the subjects with failure the greatest (Fig. 11).

C. *Potassium acetate:* Six grams of potassium acetate (61 meq. potassium) per day was administered orally to control Subject 501 and Subject 502 with moderate congestive heart failure for five and three days, respectively (Figs. 5 and 7). This resulted in a slightly more positive potassium balance in both subjects. There was a slight increase in potassium clearance but there were no other significant changes in the other time-course curves shown in Figs. 5 and 7.

D. *Sodium bicarbonate:* Ten grams (119 meq.) of sodium bicarbonate per day was administered orally to control Subject 501 (Fig. 5), Subject 502 (Fig. 7) with moderate congestive heart failure, and Subject 503 (Fig. 9) with severe congestive heart failure. The daily sequence of administration varied for these subjects as indicated by Figs. 5, 7, and 9. The administration of sodium bicarbonate to the control subject resulted in an increase in the excretion and clearance rate of sodium with a positive balance of sodium and an associated decrease in excretion of chloride and a slight positive chloride balance without a change in body weight. Upon discontinuing sodium bicarbonate, the excretion of chloride increased, again without change in body weight. Calculated chloride space increased with the retention of chloride and later decreased during the period of negative chloride balance. This is in keeping with an intracellular shift of chloride during the period of sodium bicarbonate administration and an extracellular shift after discontinuance of the sodium bicarbonate. The same was true for Subject 502 with moderate congestive heart failure. The subject with severe congestive heart failure, Subject 503, showed almost quantitative retention of the administered sodium with relatively little change in the excretion of chloride. This patient was moribund at the time of administration of the drug.

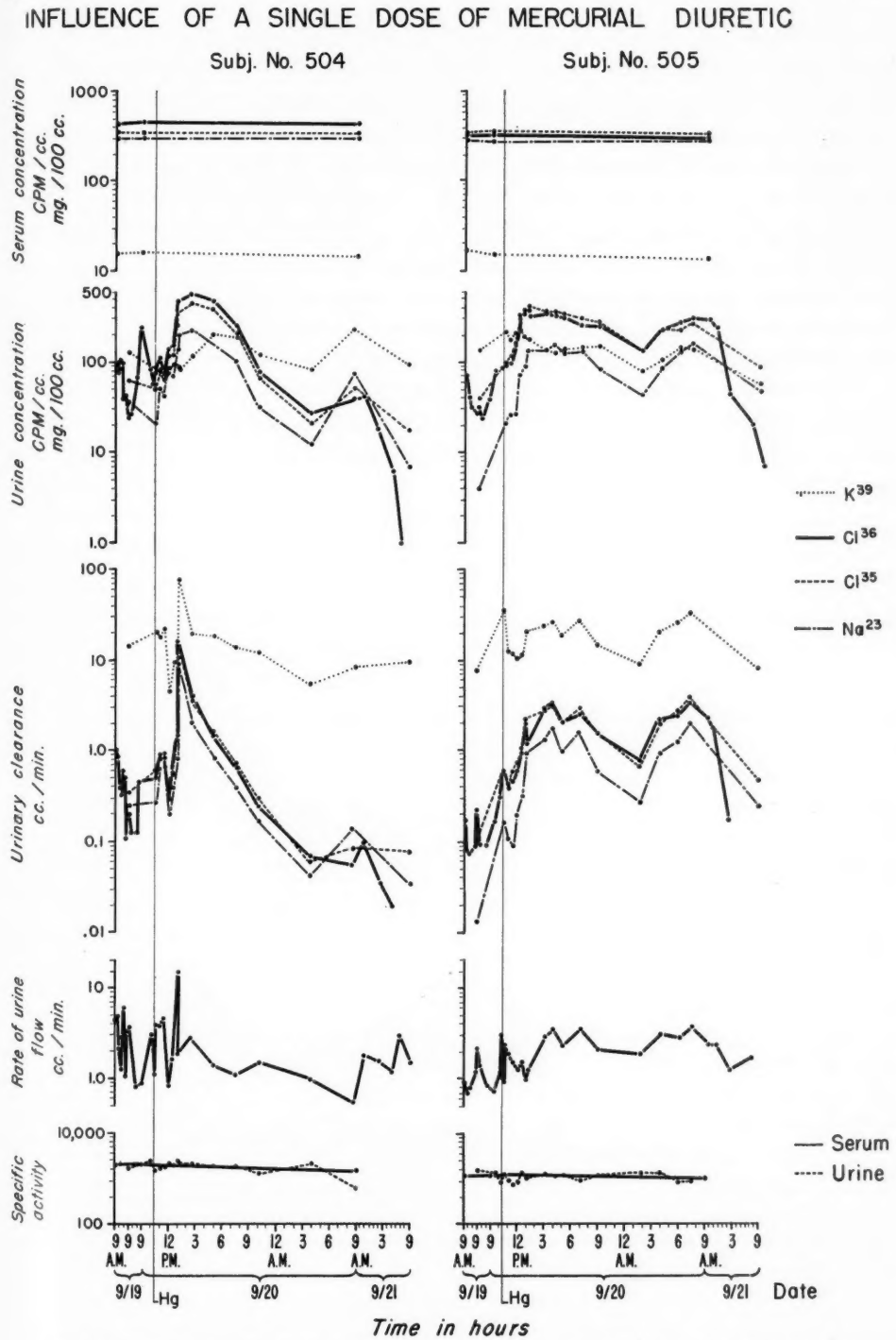

Fig. 17.—The influence in two subjects of a single dose of a mercurial diuretic (Mercuhydrin) upon serum and urine concentration and urinary clearance of Cl^{36}, Cl^{35}, sodium, and potassium as well as upon the rate of urine flow and the specific activity of Cl^{36} in both serum and urine. The subject with moderately severe congestive failure experienced a more prolonged effect from the diuretic than did the control subject.

E. *Cortisone:* Cortisone was administered intramuscularly for four and seven days, respectively, to control Subject 504 and Subject 505 with moderate congestive heart failure (Figs. 6 and 8). Each subject received 300 mg. for the first day, 200 mg. the second day, and 100 mg. each succeeding day of administration. No significant effect attributable to the cortisone could be identified.

F. *Desoxycorticosterone acetate:* Ten milligrams of desoxycorticosterone acetate in oil was administered subcutaneously to control Subject 501 and Subject 502 with moderate congestive heart failure (Figs. 5 and 7). The subjects were receiving 10 Gm. of sodium chloride per day orally in addition to the dietary intake. The DOCA was followed by a decrease in excretion and clearance of sodium and chloride and a positive balance of these elements in the control Subject 501. No definite effect on potassium was demonstrable. Subject 502 with moderate congestive heart failure showed no demonstrable effect of the DOCA.

DISCUSSION

Three ratios were employed in the analysis of the data obtained in these experiments. These ratios and their derivation are as follows: (1) The ratio of excretion of Cl^{36} as a percentage of that present in the body to excretion of Cl^{35} as a percentage of that present in the body is:

$$\frac{\dfrac{U_{36}V}{N_T} \times 100}{\dfrac{U_{35}V}{B_{36} \times B_{35}} \times 100} = \frac{U_{36}B_{35}}{U_{35}B_{36}} = 1$$

where U_{36} = urinary concentration of Cl^{36}, CPM/ml.,
U_{35} = urinary concentration of Cl^{35}, meq./ml.,
V = volume of urine per unit of time,
B_{36} = concentration of Cl^{36} in blood serum, CPM/ml.,
B_{35} = concentration of Cl^{35} in blood serum, meq./ml., and
N_T = CPM of Cl^{36} remaining in the body at a given time.

(2) The ratio of urinary clearance of Cl^{36} to urinary clearance of Cl^{35} is:

$$\frac{\dfrac{U_{36}V}{B_{36}}}{\dfrac{U_{35}V}{B_{35}}} = \frac{U_{36}B_{35}}{U_{35}B_{36}} = 1$$

(3) The ratio of specific activity of the urine to the specific activity of blood serum is:

$$\frac{\dfrac{U_{36}}{U_{35}}}{\dfrac{B_{36}}{B_{35}}} = \frac{U_{36}B_{35}}{U_{35}B_{36}} = 1$$

Data obtained from the measurements of the urine and blood concentrations of Cl^{36} and Cl^{35} may be tested for their accuracy by the use of these ratios. Whenever there is a steady state, equilibrium of distribution and a homogenous physiologic system, these ratios should equal 1. When the values are other than 1, there is a disturbance in these conditions or errors in measurement. A study of these ratios showed them to be equal at all times and to be equal to 1 when a steady state existed.

There was a delay of one to seven minutes in the appearance of the tracer in the urine following the intravenous injection. Such delay can have considerable significance whenever clearance studies are conducted for short periods of time, especially when the concentration of the substance being traced is changing rapidly in the serum or urine. These experiments, therefore, show that lag in appearance exists for Cl^{36} as well as for Hg^{203} and Na^{24} as indicated previously.[9, 10] When lags and diurnal variations in intake, rates of metabolism, and associated exchange among various chloride compartments in the body exist, in addition to the delay in appearance time in the urine, errors can become even greater and are more likely to manifest themselves and significantly modify the accuracy of the interpretations of the calculations of mass of Cl^{35}, space of Cl^{36}, clearance rates, and specific activity obtained with the use of Cl^{36}. The mechanism by which the factors might participate in exchange phenomena and production of erroneous calculated data have been discussed previously.[2, 3]

If intake of Cl^{35} is constant, the larger the Cl^{35} mass and space, the slower the rate of turnover of Cl^{35}.[3, 12] Unfortunately, it is impossible to estimate the quantity of extracellular water or Cl^{35} mass in the normal or edematous subject in order to make corrections for body size. This is further complicated in that Cl^{35} has been shown to exist in fairly large quantities in cells and to shift between extracellular and intracellular fluids. Therefore, the calculations without corrections in body weight reported earlier in this paper are subject to error and are only estimations of the true situation. In spite of this, the results show a remarkable relationship (Figs. 3 and 12).

Although chloride and sodium have been shown to enter the cellular compartment,[1, 2, 3, 11, 12] the major portion of these elements is in the extracellular fluids. The effects of lags in transfer rates and in functional sequestration of isotopic tracers have been described in detail elsewhere.[1-3] However, these effects are minimized by sampling the major compartment of these isotopes, the extracellular fluids. If the major portion of an element or isotope, such as potassium or rubidium, is within the cellular compartment, sampling the extracellular fluids may not reflect all kinetic phenomena, particularly of the major mass of the tracer and nontracer. In the instances where the major portion of an element is intracellular, that compartment must be studied just as the major or extracellular compartment was sampled in these observations on chloride.

Because of the difficulty of measuring Cl^{35} in the stools, only Cl^{36} excretion was measured in these experiments. This was prompted also in part by the general impression that only a small part of all chloride eliminated is excreted

in the stools. The two control subjects lost 2 and 4 per cent, respectively, of the total radiochloride excreted in their stools, whereas the subjects with congestive heart failure lost as much as 11 per cent of the total Cl^{36} excreted in the feces (Table II). This loss was increased in diarrheal stools. This is well demonstrated by the highest fecal loss of radiochloride in the control Subject 501 which followed the administration of Phospho-soda* (Fig. 5). This agent produced the same effect in a patient (Subject 502) with moderate congestive heart failure.

There tracer studies with the long-life isotope Cl^{36} revealed a number of metabolic phenomena of chloride which were not heretofore satisfactorily approachable with studies using Cl^{35} and Cl^{38}. Among these was the observation that with the administration of sodium bicarbonate the excretion of chloride decreased with an apparent shifting of chloride into compartments other than serum. Furthermore, subjects with congestive heart failure were unable to maintain as constant a level of concentration in their serum as the control subjects. The marked variations in concentration of radiochloride in serum could be explained only by shift of chloride from extracellular fluid into other compartments. This inability to maintain a steady chemical state of the "milieu interior" is an expression of the diffuse metabolic disturbance in electrolytes associated with congestive heart failure. This instability of chemical environment in the subjects with severe congestive heart failure was manifested by the "overreaction" to mercurial diuretics with depletion of body electrolytes and to removal of ascitic or pleural fluid. It is also noted that the excretion and the turnover rates of chloride for the body as a whole can be accelerated in direct proportion to the dietary intake of the ordinary chloride, provided that the renal function is adequate. Furthermore, with impairment of cardiac function and progression of the state of congestive heart failure, the rate of chloride turnover decreased, and with the appearance of compensation and diuresis the turnover often exceeded the turnover of normal subjects on comparable intake of chloride. It was also possible with Cl^{36} to determine fairly accurately the appearance time of Cl^{36} in the urine following the intravenous administration of the tracer and to study the influence of the lag in excretion of the tracer on estimation of urinary clearance.

The percentage of chloride present in the body that was excreted over variable periods of time, determined through the medium of long-life radiochloride, could not have been measured directly with ordinary chloride or Cl^{38}. The marked diurnal and other variations in turnover rates and rates of excretion of chloride in the urine were also more clearly defined through use of Cl^{36}. A knowledge of the biologic decay rates and influencing factors has connotations of importance not only in clinical medicine but also in atomic warfare and injuries due to radiochloride. By indirect inference the information gathered through the use of Cl^{36} concerning Cl^{35} metabolism can be applied for therapeutic purposes to bromidism and the excretion of bromide.

*Fleet's Phospho-soda.

SUMMARY

The excretion patterns of Cl^{36} have been studied in six subjects immediately following and for prolonged periods (thirty to seventy days) after a single rapid intravenous administration of 50 μc of the tracer. The concentration of Cl^{35}, sodium, and potassium was determined in the serum, urine, and other biologic fluids, and the volume of urinary output recorded. The stools were assayed only for Cl^{36} excretion.

The time of appearance of Cl^{36} in the urine following intravenous injection was found to vary from two to seven minutes in the five subjects. The rate of urinary excretion of chloride during the first ninety minutes was much less for the subjects with congestive heart failure than for the control subjects. There was an inverse relationship between the rate of excretion and the body weight of the patient, the relationship being different for the subjects with severe congestive heart failure than for the control subjects and subjects with mild or moderate congestive heart failure.

From 95 to 99 per cent of the injected tracer was recovered from the three subjects who were studied for seventy days. This is significant in the determination of the quantity of radiation to the tissues of man from Cl^{36}. Furthermore, this shows that no significant amount of Cl^{36} is "permanently" bound in compartments that fail to communicate with the exchanging compartments and subject to the excretory action of the kidneys because most of the recovered chloride was in the urine. Incidentally, 1 to 4 per cent was recovered in the stools of the control subjects.

The patterns of daily excretion of Cl^{36} in the urine and stool were presented and indicated that variations in excretion of chloride may be produced by changes in the intake of the electrolyte, administration of sodium bicarbonate, mercurial diuretics, and other drugs, or changes in the physiologic state. It was noted that the subjects with congestive heart failure manifested a greater excretion of chloride after mercurial diuretics and maintained this increased rate of excretion longer than the control subjects who showed a "rebound" retention of Cl^{35} and Cl^{36} the day following the administration of the diuretic. On both low and high chloride diets, the control subject excreted more chloride than the subjects with congestive heart failure except during periods when the latter subjects were in the process of compensation or massive diuresis initiated by either therapeutic or spontaneous factors.

There were certain discrepancies noted between the rates of excretion of Cl^{36} and Cl^{35} which have been discussed in previous reports relating to the phenomena of lags in rates of exchange and sequestration of chloride. These discrepancies have been shown to exist also between the specific activity of chloride in the urine and serum which may be explained by the same phenomena. The ratios of excretion of Cl^{36} to excretion of Cl^{35} and the specific activity in the urine to the specific activity in the serum should be equal to each other by the nature of the calculations. When they are not, errors in the selection of

data for analysis or in the analysis itself must have existed; however, deviation of these ratios from 1 can be explained by physiologic phenomena of lags and sequestration.

It has also been demonstrated that, even when the clearance rates of sodium and potassium were corrected for differences in intake and serum concentration, they were not equal to the urinary clearance of chloride. A notable discrepancy was obtained during the period of ammonium chloride administration. The corrected sodium clearance exceeded that of chloride indicating that relative to dietary intake a greater amount of sodium is being excreted. During sodium bicarbonate administration the same pattern was present because of increased sodium excretion and decreased chloride excretion.

The daily balance data for chloride, sodium, and potassium showed the patterns to conform with the observations concerning the excretory rates. When the daily balance was accumulated over long periods of time, the net balance figures were positive for most subjects, indicating that inaccuracies existed, probably due in part to estimations of intake calculated from tables of electrolyte content of foods as well as the loss of electrolytes by routes not studied.

The hourly variations following the administration of 7.5 Gm. of sodium chloride indicated that the subjects with congestive heart failure were not able to excrete administered salt as readily as the control subjects. This observation and the relationship noted to exist for the immediate excretion of Cl^{36} and body weight suggest the possibility of the development of electrolyte excretory function tests as indicators of cardiorenal function in congestive heart failure.

Finally, it is quite obvious that many aspects of Cl^{35} metabolism already known have been verified or elucidated through these studies of Cl^{36} metabolism. Because of the ease with which Cl^{36} measurements can be made in biologic fluids of many types, it was possible in these studies to make many and frequent measurements of Cl^{36} in red cells, serum, pleural fluid, stools, sputum, and sweat.

REFERENCES

1. Ray, C. T., Burch, G. E., and Threefoot, S. A.: Biologic Decay Rates of Chloride in Normal and Diseased Man, Determined with Long-Life Radiochloride, Cl^{36}, J. LAB. & CLIN. MED. **39**: 673-696, 1952.
2. Threefoot, S. A., Burch, G. E., and Ray, C. T.: Chloride "Space" and Total Exchanging Chloride in Man Measured With Long-Life Radiochloride, Cl^{36}, J. LAB. & CLIN. MED. **42**: 16-33, 1953.
3. Burch, G. E., Ray, C. T., and Threefoot, S. A.: Some Theoretic Considerations of Electrolyte Space Measured by the Tracer Method in Intact Man, J. LAB. & CLIN. MED. **42**: 34-57, 1953.
4. The Canned Food Reference Manual, ed. 2, Rogers-Kellog-Stillson, R. W. Pilcher, Editor in chief, New York, 1943, American Can Co.
5. Love, W. D., and Burch, G. E.: Plasma and Erythrocyte Sodium and Potassium Concentrations in a Group of Southern White and Negro Blood Donors, J. LAB. & CLIN. MED. **41**: 258-267, 1953.
6. Schales, O., and Schales, S.: A Simple and Accurate Method for the Determination of Chloride in Biologic Fluids, J. Biol. Chem. **140**: 879-884, 1941.
7. Burch, G. E., Ray, C. T., and Threefoot, S. A.: Estimation of the Time of Equilibrium of Distribution of Long-Life Radiochloride and Radiosodium in Man With and Without Chronic Congestive Heart Failure, Acta med. scandinav. **142**: 329-341, 1952.
8. Burch, G. E., Reaser, P. B., Ray, C. T., and Threefoot, S. A.: A Method of Preparing Biologic Fluids for Counting of Radioelements, J. LAB. & CLIN. MED. **35**: 626-630, 1950.

9. Burch, G. E., Threefoot, S. A., and Reaser, P. B.: Some Aspects of Renal Excretion of Na^{24} by Normal Subjects and by Patients With Congestive Heart Failure, Stanford M. Bull. **6**: 81-87, 1948.
10. Milnor, Pervis, Burch, George, Ray, Thorpe, Threefoot, S., and Berenson, Gerald: Considerations of Renal, Hepatic, and Extremital Arteriovenous Differences in Concentration of Radiomercury of a Mercurial Diuretic, J. Clin. Invest. **29**: 72-86, 1950.
11. Threefoot, S., Burch, George, and Reaser, Paul: The Biologic Decay Periods of Sodium in Normal Man, in Patients With Congestive Heart Failure, and in Patients With the Nephrotic Syndrome as Determined by Na^{22} as the Tracer, J. LAB. & CLIN. MED. **34**: 1-13, 1949.
12. Burch, G. E., Threefoot, S. A., and Cronvich, J. A.: Theoretic Considerations of Biologic Decay Rates of Isotopes, J. LAB. & CLIN. MED. **34**: 14-30, 1949.
13. Marinelli, L. D., Quimby, Edith H., and Hine, G. J.: Dosage Determination With Radioactive Isotopes. II. Practical Considerations in Therapy and Protection, Am. J. Roentgenol. **59**: 260-281, 1948.
14. Pitts, R. F., and Axelrod, D. R.: A Chloride Bicarbonate Shift in Body Cells, Presented at the American Physiologic Society, Sept. 4, 1952, at New Orleans, La.
15. Sims, E. A. H., Welt, L. G., Orloff, J., and Needham, J. W.: Asymptomatic Hyponatremia in Pulmonary Tuberculosis, J. Clin. Invest. **29**: 1545-1557, 1950.
16. Verney, E. B.: Absorption and Excretion of Water. The Antidiuretic Hormone, Lancet **2**: 781, 1946.
17. Burch, George, Ray, Thorpe, Threefoot, S., Kelly, F., and Svedberg, A.: The Urinary Excretion and Biologic Decay Period of Radiomercury Labeling a Mercurial Diuretic in Normal and Diseased Man, J. Clin. Invest. **29**: 1131-1138, 1950.

THE UTILIZATION OF AMINO ACID NITROGEN FOLLOWING TRAUMA

II. Further Studies

Richard L. Burt, Ph.D., M.D.
Winston-Salem, N. C.
With the Technical Assistance of C. A. Kimel and H. R. Fallers, Jr.

INTRODUCTION

In a previous study[1] it was reported that amino nitrogen retention in the plasma following parenteral injection of L-lysine monohydrochloride occurred in the dog after trauma (laparotomy). This phenomenon was characterized by retardation of the rate of disappearance of intravenously administered amino nitrogen in the plasma, the defect persisting for about ten days following operation. During the postoperative period there was a gradual return to normal rates of utilization. On the basis of results showing apparently normal liver function (as tested by bromsulphalein), unaltered plasma protein concentration, and failure to induce the defect by premedication and anesthesia in the absence of surgical injury, it was suggested that such a physiological defect may have resulted from impairment of the normally rapid rate of tissue fixation of the amino nitrogen which was originally described by Van Slyke and Meyer.[2] This report is concerned with further studies of plasma amino nitrogen utilization following injury.

EXPERIMENTAL

Adult mongrel dogs in apparent good health were employed throughout. Animals received from the pound were held for a period of approximately two weeks prior to experiment during which time they were deparasitized and observed for any evidence of infection. Purina or Pillsbury's dog chow was used as the basic ration with supplements of raw meat. No effort was made to control or quantitate dietary intakes. Laparotomy was performed under Nembutal anesthesia with morphine-atropine premedication.[3] All incisions healed per primum. Certain dogs were "conditioned" by maintenance at kennels with added exercise periods and more liberal meat supplements daily for periods of two weeks prior to experiment.

As previously described,[1] each dog served as its own control, loading curve determinations being obtained during the preoperative and postoperative periods. Following the withdrawal of venous blood from the foreleg, intravenous injection of DL-alanine, L-lysine monohydrochloride, or casein hydrolysate in 0.9 per cent saline was made in the saphenous

From the Department of Obstetrics and Gynecology of the Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

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vein. Blood samples were collected with minimal stasis at five- and ten-minute intervals following parenteral administration of the amino acid preparation. Connaught heparin was routinely employed as anticoagulant. In additional experiments, serum rather than plasma was used for the loading tests.

Plasma protein concentration was determined by the method of Phillips and associates.[4] The ninhydrin-manometric method was used for the determination of free alpha amino nitrogen in plasma.[5, 6] Values are considered accurate to ± 1 per cent at the levels of amino nitrogen encountered. Alanine nitrogen was determined in tungstic acid filtrates with an accuracy of ± 0.05 mg. per cent by the method of Alexander and Seligman[7] as modified by Christensen.[8] Plasma urea nitrogen was estimated by a steam distillation technique.[1]

The effect of larger doses of L-lysine monohydrochloride on postoperative amino nitrogen retention of the plasma was tested (6.0 and 10.0 mg. alpha amino nitrogen per kilogram of body weight). A dispensable amino acid (DL-alanine) was employed to determine possible differences in its rate of utilization following injury as contrasted to lysine. In addition, in certain loading curve experiments the concentration of alanine-N was determined after DL-alanine administration. As the parenteral use of a single crystalline amino acid might be considered unphysiological from a nutritional standpoint, a casein hydrolysate preparation was employed in additional experiments.

Five animals were operated upon for the second time after a suitable convalescent period of one to two months and loading curve determinations were again performed to test response after repeated injury. In an additional experiment, one animal was studied before and after operation with regard to blood urea nitrogen levels following the administration of L-lysine monohydrochloride (6.0 mg. alpha amino nitrogen per kilogram). To estimate the role of dietary restriction during the postoperative period as a possible factor in the production of plasma amino nitrogen retention, three dogs were starved for 120 hours and loading experiments were performed daily with 3.0 mg. per kilogram alpha amino nitrogen as L-lysine monohydrochloride.

RESULTS

The results obtained with lysine (Table I) show that following laparotomy there was a disturbance in the rate of utilization of total alpha amino nitrogen which was most marked within the first forty-eight hours as was originally reported for lower dosage levels.[1] This is indicated by a larger increment in the postoperative ten-minute plasma values as contrasted to those obtained during the preoperative control periods. Within the dosage limits used, increase in the amount of amino acid injected did not result in proportionately higher plasma amino nitrogen levels either pre- or postoperatively. With larger doses, however, the defect in utilization after injury was apparent for somewhat longer periods. The results with 6.0 and 10.0 mg. per kilogram doses were quite similar. In subsequent observations with lysine, as well as alanine, some variation in the degree of postoperative amino nitrogen retention as well as variance in the persistence of this alteration in the rate of utilization have been observed. Although the response to loading tests with DL-alanine at levels between 4.0 and 10 mg. per kilogram did not differ significantly from the results obtained with lysine, "retention" was not found for casein hydrolysate (Table I). Total plasma protein concentration was not found to be affected by the surgical procedure employed. Except for slightly higher individual values for amino nitrogen, the studies on serum did not vary significantly from those in which plasma was employed.

TABLE I. EFFECT OF LAPAROTOMY ON PLASMA AMINO NITROGEN RETENTION FOLLOWING ADMINISTRATION OF 10.0 MG. PER KILOGRAM ALPHA AMINO NITROGEN

L-LYSINE MONOHYDROCHLORIDE					CASEIN HYDROLYSATE				
DOG NO.	PRE- OR POST-OPERATIVE DAY	PLASMA AMINO NITROGEN (MG. PER 100 ML.) 0 MIN.	5 MIN.	10 MIN.	DOG NO.	PRE- OR POST-OPERATIVE DAY	PLASMA AMINO NITROGEN (MG. PER 100 ML.) 0 MIN.	5 MIN.	10 MIN.
6	13	4.57	6.11	5.01	9	7	4.80	8.16	6.82
M	7	4.69	6.29	5.19	F	5	4.60	6.15	5.55
	4	4.56	5.87	5.30		1	4.53	5.96	5.35
	Laparotomy								
	2	4.64	5.65	5.94		2	4.34	6.38	5.60
	4	4.53	5.79	5.95		4	4.56	7.42	7.46
	7	4.63	5.71	5.78		7	4.69	7.35	6.19
	9	4.73	6.48	6.20		9	4.54	6.45	5.73
	14	4.68	6.11	5.75					
9	13	4.47	5.70	4.92	20	7	4.94	7.04	6.04
F	7	4.35	5.55	4.80	M	5	4.78	6.74	6.25
	4	4.37	5.71	5.00		1	5.03	6.57	5.83
	Laparotomy								
	2	4.49	5.68	5.81		2	4.84	6.41	5.68
	4	4.55	5.37	5.69		4	4.81	6.47	5.91
	7	4.32	5.72	5.68		7	4.92	6.67	5.72
	9	4.40	5.88	5.72		9	4.98	6.68	5.98
	14	4.44	5.90	5.63					
21	13	4.50	5.96	5.08	21	7	4.38	6.42	5.42
M	7	4.39	6.00	5.22	M	5	4.51	6.26	5.28
	4	4.26	5.64	5.08		1	4.28	6.47	5.51
	Laparotomy								
	2	4.34	5.38	5.66		2	4.19	6.36	5.88
	4	4.52	5.52	5.68		4	4.33	6.31	5.73
	7	4.51	6.04	6.02		7	4.41	6.97	5.73
	9	4.49	5.86	5.68		9	4.38	6.80	5.37
	14	4.59	6.02	5.66					

In one animal blood urea nitrogen levels before as well as after operation were found to vary within narrow limits during the ten-minute period following injection of 6.0 mg. per kilogram alpha amino nitrogen as L-lysine monohydrochloride. After a suitable recovery period amino nitrogen retention in the plasma was again demonstrated in five dogs subjected to second laparotomy.

Results of loading determination made with L-lysine monohydrochloride during starvation (3 mg. alpha amino nitrogen per kilogram) are shown in Table II. Fasting up to 120 hours was not productive of amino nitrogen retention in the plasma. During this period the total protein concentration of plasma and the fasting plasma amino nitrogen values were quite constant. Each aimal, however, lost about two kilograms in body weight. We have regularly observed similar invariability of fasting plasma amino nitrogen and plasma protein concentration throughout control and experimental periods although the animals usually incurred a small weight loss during the immediate postoperative period.

The results of loading curve determinations with DL-alanine between 5 and 10 mg. per kilogram as alpha amino nitrogen before and after laparotomy are

TABLE II. EFFECT OF STARVATION ON PLASMA AMINO NITROGEN RETENTION FOLLOWING PARENTERAL INJECTION OF 3.0 MG. PER KILOGRAM ALPHA AMINO NITROGEN (L-LYSINE MONOHYDROCHLORIDE)

DOG NO.	DAYS OF FAST*	BODY WEIGHT (KG.)	PLASMA AMINO NITROGEN (MG. PER 100 ML.) 0 MIN.	5 MIN.	10 MIN.
15		24.0	4.55	5.45	4.90
M	1	24.1	4.62	5.46	4.80
	2	23.4	4.35	5.46	4.66
	3	23.2	4.53	5.57	5.19
	4	22.7	4.41	5.46	5.09
	5	22.2	4.37	5.55	4.99
19		21.7	3.55	4.54	3.96
M	1	21.1	3.69	4.60	4.06
	2	20.7	3.58	4.65	4.05
	3	20.6	3.43	4.75	4.01
	4	20.3	3.32	4.60	4.07
	5	19.7	3.36	4.35	4.01
23		21.8	5.47	6.50	5.75
M	1	22.9	5.57	6.53	6.15
	2	21.8	5.36	6.42	5.87
	3	21.6	5.45	6.40	5.69
	4	21.3	5.46	6.36	5.87
	5	20.6	5.30	6.37	6.05

*Broken line indicates time interval of 1 week.

shown in Table III. It is apparent that at the dosage levels of alpha amino nitrogen employed, trauma tended to retard the normal decrease in alanine nitrogen concentration of plasma in the five- and ten-minute interval following administration. This effect was not found, however, for Dog 7A in which the five- to ten-minute decrement in plasma amino acid concentration was actually greater following the surgical procedure (absence of retention). "Conditioned" animals exhibited no essential difference in response to loading after laparotomy.

DISCUSSION

In the original study concerning the effect of trauma on the rate of disappearance of amino acid nitrogen following parenteral administration in dogs it was found that consistent and significant retention of total free alpha amino nitrogen was characteristic of the early posttraumatic state.[1] The present study confirms the essential features of the original observations, although variability in both the degree and occurrence of amino nitrogen retention has been observed. With special reference to the effect of trauma on the plasma concentration of the individual amino acid DL-alanine it was found that not only is total amino-nitrogen retained as previously described, but this change is associated with a parallel delay in the disappearance of alanine-N itself.

The absolute amount of amino nitrogen retained in the plasma following parenteral administration of lysine is not found to be proportional to the dose of the amino acid given, however, either before or after trauma. Increments

TABLE III. EFFECT OF DL-ALANINE LOADING BEFORE AND FOLLOWING LAPAROTOMY

DOG NO.	PREOPERATIVE OR POSTOPERATIVE DAY	ALANINE-N (MG. PER 100 ML. PLASMA) 0 MIN.	5 MIN.	10 MIN.	5-10 MIN.Δ
1A*	18	0.79	2.04	1.89	−0.15
M	13	0.16	2.17	1.76	−0.41
	7	0.41	2.26	1.86	−0.40
	Laparotomy				
	2	0.32	1.95	2.58	+0.63
	4	0.47	1.95	1.76	−0.19
	6	0.35	1.79	1.57	−0.22
2A*	14	0.25	2.01	1.60	−0.41
M	7	0.19	1.76	1.54	−0.22
	3	0.18	1.89	1.29	−0.60
	Laparotomy				
	1	0.28	1.82	1.94	+0.12
	3	0.28	1.60	1.42	−0.18
	4	0.28	2.01	1.32	−0.69
3A†	6	0.72	7.63	4.88	−2.75
M	4	1.29	6.45	4.96	−1.47
	Laparotomy				
	1	0.50	8.65	8.81	+0.16
	3	0.50	1.97	1.26	−0.71
4A*	9	0.44	1.60	1.25	−0.35
M	6	0.69	1.79	1.38	−0.41
	Laparotomy				
	2	0.63	1.60	1.51	−0.09
	3	0.63	1.89	1.45	−0.44
7A*	6	0.63	2.36	1.89	−0.47
M	2	0.63	1.57	1.32	−0.25
	Laparotomy				
	1	0.66	2.86	1.73	−1.13
	2	0.47	1.82	1.26	−0.56

*5 mg. per kilogram alpha-amino-N
†10 mg. per kilogram alpha-amino-N

proportional to dosage, as reported by Silber and associates[9] for amino acid mixtures, might have been obtained by use of a continuous infusion technique with greater total amounts of amino acid per loading determination.

The maintenance of increased plasma amino nitrogen values following loading as indicative of retention does not necessarily involve the assumption that the increase is derived solely from the injected material. Analyses of individual amino acids in the plasma after administration indicate that fairly rapid interconversion may occur[10-12] and the release of other amino acids by the tissues in response to the injection of a single compound or unbalanced amino acid mixture conceivably could contribute to the amino nitrogen values determined. Contrary to the latter view, however, is the study of Hier[12] showing that the injection of leucine, iso-leucine, or methionine produced a significant decrease in the concentration of certain amino acids in the plamsa, although the

effect of alanine and lysine was not investigated. The fact that apparent utilization of DL-alanine as well as L-lysine is disturbed by trauma raises the question as to whether the defect described is limited to individual or to certain combinations of amino acids, a definitive answer to which must await further studies.

Our failure to demonstrate a consistent posttraumatic amino-nitrogen retention with casein hydrolysate, in contrast to lysine and alanine, might be interpreted as indication of normal utilization of the hydrolysate preparation following injury. On the other hand, it is possible that defective utilization of certain constituent amino acids in the mixture may have escaped detection inasmuch as total amino nitrogen rather than individual amino acid concentrations in the plasma was measured. In the absence of further evidence, a conclusive explanation of the dissimilar responses of individual amino acids and casein hydrolysate after injury is not possible.

The method employed for the determination of alpha amino nitrogen in the plasma requires that the values obtained in a given analysis be corrected for blood urea nitrogen,[5] this correction amounting to about minus 0.1 mg. per 100 ml. plasma. The demonstration in one animal that blood urea nitrogen did not vary appreciably during the loading experiments tends to exclude increases in urea nitrogen as accounting for the posttraumatic amino nitrogen retention described. This is consistent with the data of Goettsch and associates[13] showing only small increments in blood urea nitrogen following the administration of somewhat larger doses of amino nitrogen than were employed in our studies.

The absolute amount of amino nitrogen retained in the plasma in consequence of injury constitutes but a small percentage of the total amount administered in the individual loading tests. As determined by the Theory of Runs,[24] however, the combined probability of the differences between the five- and ten-minute levels arranging themselves in the fashion observed (Table I) is much less than 0.01. This retention may be related to the known disturbance of total nitrogen metabolism resulting from trauma.[17, 18] From calculations based on data of Gibson and his co-workers[14] of an estimated plasma volume of 5.4 per cent of the body weight, between 80 to 95 per cent of the injected amino nitrogen is removed from the circulating plasma within five minutes, a finding consistent with the data obtained by other workers for certain amino acids and amino acid mixtures.[2, 15, 16]

In this study no attempt is made to maintain the animals on an isocaloric dietary, nor were quantitative determinations of food consumption made during the experimental periods. Our data, however, show that starvation up to five days had no apparent effect on the utilization of amino nitrogen administered as L-lysine monohydrochloride. This observation appears to exclude a dietary influence in production of amino nitrogen retention following surgical injury and contrasts with the data of White and associates[20] who reported an increased rate of incorporation by the tissues of N-15 as glycine in fasting mice. No significant change in plasma amino nitrogen occurred during the five-day starvation period, a finding consistent with the earlier observation of Van Slyke and Meyer.[2]

Although physical condition and nutritional status appear to be related to posttraumatic nitrogen wastage in the human being,[18, 21] additional activity and higher protein intakes were without effect on amino nitrogen retention under the conditions of these experiments. Moreover, the release of amino acid nitrogen by the clotting mechanism of blood[22] cannot account for the results obtained in loading curve determinations as evidenced by failure to obtain increased retention when serum rather than plasma was employed. The variable degrees of retention occasionally observed suggest that other factors are present which can affect the posttraumatic utilization or tissue fixation of amino acids, factors that may bear on the occurrence of variance in total nitrogen metabolism in association with traumatic states in the human being.[18, 21] The diversity of alternative metabolic pathways available for the utilization of alanine[23] suggests the complexity of the problem of nitrogen wastage (metabolism) in response to trauma and may be related to the variation in the degree of alanine-N retention which we have observed in the posttraumatic state.

SUMMARY

1. In extension of a previous report, posttraumatic nitrogen retention has been demonstrated with increasing doses of L-lysine monohydrochloride. The degree of retention was not found to be proportional to the dose of amino acid administered, and the absolute amount of retained amino nitrogen was small. The effect could be repeatedly demonstrated in the same dog following a suitable postoperative recovery period.

2. Similar retention of amino nitrogen in plasma after injury has been found with DL-alanine, but not with a casein hydrolysate preparation. Determinations of alanine nitrogen per se suggest that the amino nitrogen retention observed is perhaps accountable on the basis of defective utilization of the individual amino acid administered.

3. Starvation for the period of 120 hours had no significant effect on the plasma amino nitrogen concentration or on amino nitrogen loading responses.

4. As previously observed, operative trauma was without effect on the postabsorptive plasma amino nitrogen concentration.

REFERENCES

1. Burt, R. L., and Fallers, H. R., Jr.: Amino Nitrogen Retention in Plasma Following Trauma, J. Biol. Chem. **177**: 825, 1949.
2. Van Slyke, D. D., and Meyer, G. M.: The Amino Acid Nitrogen of the Blood. Preliminary Experiments on Protein Assimilation, J. Biol. Chem. **12**: 399, 1912.
3. Seevers, M. H.: Morphine-pentobarbital Anesthesia for Dog Surgery, J. LAB. & CLIN. MED. **19**: 202, 1933.
4. Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., Dole, V. P., Emerson, K., Jr., and Archibald, R.: Measurement of Specific Gravities of Whole Blood and Plasma by Standard Copper Sulfate Solutions, J. Biol. Chem. **183**: 305, 1950.
5. Hamilton, P. B., and Van Slyke, D. D.: The Gasometric Determination of Free Amino Acids in Blood Filtrates by the Ninhydrin-carbon Dioxide Method, J. Biol. Chem. **150**: 231, 1943.
6. Hamilton, P. B., and Van Slyke, D. D.: The Effects of the Volatile Aldehydes Formed on the Accuracy of the Manometric Ninhydrin-carbon Dioxide Method in Analysis of Certain Alpha Amino Acids, J. Biol. Chem. **164**: 249, 1946.
7. Alexander, B., and Seligman, A. M.: A Colorimetric Method for the Microdetermination of Alpha Alanine in Blood, J. Biol. Chem. **159**: 9, 1945.

8. Christensen, H. N., and Lynch, E. L.: The Conjugated, Non-protein, Amino Acids of Plasma. I. Postabsorptive Concentrations of Human Plasma, Serum, and Erythrocytes, J. Biol. Chem. **163:** 741, 1946.
9. Silber, R. H., Seeler, A. O., and Howe, E. E.: Urinary Excretion of Alpha Amino Nitrogen Following Intravenous Administration of Amino Acid Mixtures, J. Biol. Chem. **164:** 639, 1946.
10. Gutman, G. E., and Alexander, B.: Studies of Amino Acid Metabolism. I. Blood Glycine and Alanine and Their Relationship to the Total Amino Acids in Normal Subjects, J. Biol. Chem. **168:** 527, 1947.
11. Christensen, H. N., Cooper, P. F., Jr., Johnson, R. D., and Lynch, E. L.: Glycine and Alanine Concentrations of Body Fluids; Experimental Modification, J. Biol. Chem. **168:** 191, 1947.
12. Hier, S. W.: Influence of Ingestion of Single Amino Acids on the Blood Level of Free Amino Acids, J. Biol. Chem. **171:** 813, 1947.
13. Goettsch, E., Lyttle, J. D., Grim, W. M., and Dunbar, P.: Amino Acid Studies. III. The Reversibility of Plasma Amino Acid Retention During Recovery From Dietary Hyproteinemia in the Dog, J. Biol. Chem. **151:** 149, 1943.
14. Gibson, J. G., Peacock, W. C., Seligman, A. M., and Sack, T.: Circulating Red Cell Volume Measured Simultaneously by the Radioactive Iron and Dye Methods, J. Clin. Invest. **25:** 838, 1946.
15. Friedberg, F., and Greenberg, O. M.: Partition of Intravenously Administered Amino Acids in Blood and Tissues, J. Biol. Chem. **168:** 411, 1947.
16. King, F. B., and Rapport, D.: The Fate of Intravenously Injected Tyrosine, Am. J. Physiol. **103:** 288, 1933.
17. Cuthbertson, D. P.: The Metabolism of Protein and Amino Acids, Ann. Rev. Biochem. **16:** 153, 1947.
18. Moore, F. D., and Ball, M. R.: The Metabolic Response to Surgery, Springfield, Ill., 1952, Charles C Thomas, Publisher.
19. Goettsch, E., Lyttle, J. D.: Grim, W. M., and Dunbar, P.: Amino Acid Studies. I. Plasma Amino Acid Retention in the Hypoproteinemic Dog as Evidence of Impaired Liver Function, J. Biol. Chem. **144:** 121, 1942.
20. White, A., Hoberman, H. D., and Szego, C. M.: Influence of Adrenolectomy and Fasting on the Incorporation of Isotopic Nitrogen Into Tissues of Mice, J. Biol. Chem. **174:** 1049, 1948.
21. Howard, J. E.: Protein Metabolism During Convalescence After Trauma, Arch. Surg. **50:** 166, 1945.
22. MacFadyen, D. A.: Determination of Amino Acids in Plasma by the Ninhydrin-carbon Dioxide Reaction Without Removal of Proteins, J. Biol. Chem. **145:** 387, 1942.
23. Potter, Van R., and Heidelberger, C.: Alternative Metabolic Pathways, Physiol. Rev. **30:** 487, 1950.
24. Swed, F. A., and Eisenhart, C.: Tables for Testing Randomness of Grouping in a Sequence of Alternatives, Ann. Math. Statist. **14:** 66, 1943.

THE EFFECT OF ALPHA METHYL STILBENE ON BLOOD SUGAR AND ON THE INSULIN CONTENT OF THE PANCREAS OF ANIMALS

MORRIS N. GREEN, PH.D., SAMUEL B. BEASER, M.D., AND
ARNOLD M. SELIGMAN, M.D.
BOSTON, MASS.

HOUSSAY[1] has recently reviewed the results of experiments regarding the effect of sex hormones in experimental diabetes. Following subtotal pancreatectomy a greater percentage of male rats than female rats developed diabetes. In explanation of these results, it was observed that the administration of estrogens stimulated the islands of Langerhans resulting in the production of new beta cells at the expense of the centro-acinar cells. These experiments pointed out the possibility of treating clinical diabetes with estrogens. The undesirable side effects of the estrogens themselves made it important to find structurally related drugs without estrogenic activity.

Griffiths and Young[2] showed that stilbesterol (4,4′-dihydroxy-α, β-diethyl-stilbene) induced a substantial increase in the insulin content of the pancreas. Griffiths[3] tested several substituted stilbenes, including α-methyl stilbene (AMS). The latter compound had previously been shown to be nonestrogenic in large doses by Dodds, Fitzgerald, and Lawson.[4] Griffiths[3] confirmed this observation. He also demonstrated that when this substance was injected subcutaneously into rabbits, a marked increase in the insulin content of the pancreas resulted. Three treated animals were found to have an average of 24.49 units of insulin per gram of pancreas, while five controls averaged 5.20 units. The insulin was assayed by the method of Marks and Pak.[5] The blood sugar values of the rabbits were essentially in the normal range.

The present communication reports observations made on rats, dogs, as well as rabbits, for changes in the insulin content of the pancreas after the injection of α-methyl stilbene. Also reported are experiments to test the effect of AMS on the glucose tolerance curves of both normal rabbits and those made diabetic with alloxan.

MATERIAL AND METHOD

Synthesis of α-Methyl Stilbene.—Benzyl magnesium chloride was allowed to react with acetophenone[6] and the resulting complex was decomposed with a saturated solution of ammonium chloride. The product of the reaction, benzyl, phenyl, methyl carbinol was extracted from the precipitated magnesium chloride with petroleum ether. The combined organic solvents containing the carbinol were removed by distillation and the carbinol was dehydrated by refluxing for five hours with glacial acetic acid containing 0.3 per cent of concentrated sulfuric acid. Upon cooling, crude α-methyl stilbene separated in crystals.

From the Yamins Laboratory for Surgical Research, Beth Israel Hospital, and the Department of Surgery, Harvard Medical School, Boston, Mass.

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The supernatant fluid was extracted with petroleum ether to recover additional crude compound. The combined crude products were recrystallized from ethanol in white platelets melting at 80° to 81° C.; uncorrected, yield 70 per cent, literature melting point 82° to 83°.[6]

Preparation of Pancreatic Extracts for Insulin Assay.—Alpha methyl stilbene was dissolved in olive oil and injected into animals subcutaneously for varying periods of time. Controls were injected for an equal length of time with the same amounts of olive oil. In some of the early experiments an insulin extraction method* (A) was employed which used evaporation under reduced pressure. This method did not give uniform results in our hands and for that reason the method (B) of Scott and Fisher[7] was used instead in later assays. Dilutions were made with U.S.P. insulin diluting fluid.[8]

The procedure for the original extraction method, although later abandoned, is given briefly below:

The pancreas was weighed and 30 ml. of 70 per cent ethyl alcohol containing 0.01 ml. of H_2SO_4 was added per gram of tissue. The mixture was quickly homogenized in a Waring Blendor and centrifuged immediately. An aliquot of the supernatant fluid was carefully measured and evaporated in a distillation flask provided with a capillary inlet of air under reduced pressure obtained with a water pump until a thick syrup was obtained. The residue was removed with a pipette; its volume carefully measured, transferred to another distillation flask, and diluted with absolute ethyl alcohol to 92 per cent by volume. The traces of residue left in the original flask were rinsed out quantitatively with 92 per cent alcohol. The total volume of the combined fluids was recorded and one and one-half volumes of ether were added. After standing overnight in the icebox the insulin precipitated out. Most of the supernatant fluid was removed with a pipette and discarded. The residue was evaporated under reduced pressure until all the alcohol was removed. The residual solution was diluted so that each milliliter contained 0.20 unit of insulin based on the assumed insulin content of the extracted pancreas.

Insulin Assay.—The extracted insulin was assayed by the method of Young and Lewis.[9] In this method mice are placed in a drum tilted at 45° and rotated with a speed of 3 r.p.m. The number of mice dropping off in one hour are counted. Mice which had received a hypoglycemic dose of insulin lost their hold on the revolving screen, dropped into metal baskets, and revived themselves by eating the food placed therein. Female mice weighing between 20 and 30 grams were used in order to minimize variation. The extreme weights in any group of mice used for a particular assay did not vary by more than 5 grams. The mice were also preselected to exclude those that dropped off even though not injected with insulin. This was done by testing each new batch of mice after eighteen hours without food, having free access to water, and excluding those that fell off during a period of one hour. In a typical assay, thirty-six fasting mice (eighteen hours) were injected subcutaneously with a single dose of the standard preparation or the preparation of unknown insulin content in a volume of 0.25 ml. per mouse. Then separate batches of mice were tested with two doses each of the standard preparation and the preparation of unknown insulin content. Standard preparations were made in which the highest dose was three times that of the lowest. A similar ratio was also employed with the unknown. The standard insulin dose per mouse was 0.0166 and 0.0500 unit. The pancreatic extracts were diluted to the same levels of activity based upon the assumed potency of the pancreatic extracts. The normal level of activity of pancreas for various species tabulated by Haist[10] was the basis for the computation of the required initial dilutions and corresponded to the values of our controls. When sufficient extracted insulin was available, assays were repeated several times. Computation of the relative potency of the unknown as compared to the standard and its statistical significance was made by the graphic method of Litchfield and Wilcoxson.[11]†

*The procedure used was modified from a procedure suggested by Dr. F. B. Peck of Eli Lilly & Company in a personal communication to Dr. Samuel B. Beaser.

†Some of the pancreases were also stained by the histochemical method of Barrnett and Seligman[13, 14] for demonstrating the disulfide groups of insulin. No differences in the intensity of staining of islet cells could be detected histochemically between the controls and the experimental animals with their method.

RESULTS

Results With Rabbits.—The initial experiments were made with a group of nine normal rabbits weighing between 1.8 and 4.5 kilograms. Five rabbits were injected with 20 mg. of AMS in 0.25 ml. fresh olive oil, five days per week for four weeks until a total of 400 mg. had been injected. A control group of four rabbits were injected with olive oil alone. In these experiments the pancreases were extracted by method A. These rabbits as well as rabbits that had neither AMS nor olive oil all showed the same level of insulin activity in their pancreases.

In another group of nine rabbits, larger doses of AMS were injected. Those weighing under 3 kilograms received 40 mg. and those weighing over 3 kilograms received 80 mg. These animals received injections five times weekly for four weeks (total 800 to 1,600 mg.) and others were injected for an additional eight weeks (total 2,400 to 4,800 mg.) before assay. Control animals were injected with olive oil alone. In a further experiment to evaluate sex differences, two male rabbits and two female rabbits each weighing 4 kilograms were injected with a total dosage of 400 mg. of AMS per week for a total of four weeks (total dose 1,600 mg.). A similar group of controls received olive oil. In this and in succeeding experiments, the insulin extraction method (B) of Scott and Fisher[7] was used. No statistically significant differences in insulin activity were noted in any of the animals.

Results With Rats and Dogs.—Ten male and ten female rats, each weighing between 200 and 250 grams, were injected twice weekly with 33 mg. of AMS in 0.25 ml. of olive oil for a period of three weeks. A similar group of controls were injected with olive oil simultaneously. Several pancreases were pooled in order to get sufficient material for an assay.

Three male and three female dogs, the former weighing an average of 8.8 kilograms and the latter, 7.7 kilograms, were injected with 200 mg. of AMS in olive oil five times weekly. Two male and two female controls were simultaneously injected with olive oil. An initial group of experimental and control animals were sacrificed after a period of three months and the last animals after nine months.

No statistically significant differences could be observed in the insulin contents of the pancreases of the treated and control animals in any of the experiments described previously.

Effect of AMS on Carbohydrate Metabolism.—Method: In the following experiments, the blood sugar method used was the Hoffman micromethod[12] modified to yield a larger filtrate by using 0.2 ml. of venous blood in 6 ml. of tungstate-precipitating solution. The AMS was given intramuscularly as a 10 per cent solution in peanut oil. Some heat was required to dissolve the AMS prior to injection.

All rabbits were nonpregnant females, 2.5 to 2.9 kilograms in weight, and were on a diet of rabbit pellets ad libitum supplemented by green vegetables. In the experiments in which alloxan was injected to produce diabetes, glucose

was added to the water for thirty-six to forty-eight hours thereafter. All glucose tolerance tests were performed with 25 per cent dextrose in distilled water injected intravenously in a dose of 0.25 Gm. per kilogram body weight and blood was taken for glucose determinations at fasting 0.5, 1, and 2 hours after injection.

Results in Normal Rabbits.—Four animals were given AMS for sixty-three days, two at a dosage level of 25 mg., and 2 at 50 mg. per day. Thereafter all four received no medication for sixty-three days, and finally all received another series of injections of 75 mg. for sixty-three more days. Glucose tolerance tests were performed before, at days 42 and 63 of the first course; at 30, 58, and 63 days in the interval between courses; and at days 21, 42, and 63 of the second course of therapy. No statistically significant alteration in the glucose tolerance test resulted from the administration of AMS.

Diabetic Rabbits.—Severe diabetes was produced by the intravenous administration of a 5 per cent aqueous solution of alloxan (150 mg. per kilogram). Many animals died, and some had a remission of their disease. Three severely diabetic animals were followed for four to eight weeks, then 50 mg. of AMS was administered daily for two months and then they were followed for a minimum of two more months. They required from 2 to 16 units of protamine zinc insulin for survival and maintenance of health. Blood sugar determinations were performed two to three times weekly.

Milder diabetes was produced by the injection of smaller doses of alloxan, 50 to 60 mg. per kilogram. With this dosage level, diabetes frequently either failed to appear or was rapidly remitted. Accordingly, repeated injections, up to four, with the same or larger (50 to 90 mg.) doses were needed to achieve the permanent diabetes. Many animals had to be discarded because excessively severe diabetes sometimes resulted. Seven rabbits thus prepared were able to continue indefinitely without insulin or ill health. Their blood sugar levels ranged from 200 to 450 mg. per cent. These animals were observed for a base period of five months. The AMS was administered at a dosage level of 50 mg. daily for twelve weeks and then observations continued for another two to three months.

In the diabetic rabbits, as measured by insulin requirements, blood sugar levels, or glucose tolerance tests, neither the severe nor the milder diabetic rabbits showed any change in their diabetes as a result of injection of AMS.

SUMMARY AND CONCLUSIONS

Under the conditions of our experiments, the injection of alpha methyl stilbene (AMS) in olive oil subcutaneously into rabbits, rats, and dogs did not cause any change in the insulin content of the pancreas contrary to results as previously reported by Griffiths.[3] No changes were observed in the glucose tolerance curves of rabbits similarly treated who had been made either severely or mildly diabetic with alloxan.

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REFERENCES

1. Houssay, B. A.: Action of the Sex Hormones on Experimental Diabetes, Brit. M. J. **2:** 505-510, 1951.
2. Griffiths, M., and Young, F. G.: Does the Hypophysis Secrete a Pancreotropic Hormone? Nature **146:** 266-267, 1940.
3. Griffiths, M.: The Effect of Derivatives of Stilbene on the Insulin Content of the Pancreas, Proc. Linnean Soc., New South Wales **67:** 279-281, 1942.
4. Dodds, E. C., Fitzgerald, M. E. H., and Lawson, W.: Estrogenic Activity of Some Hydrocarbon Derivatives of Ethylene, Nature **140:** 772, 1937.
5. Marks, H. F., and Pak, C.: Evaluation of the New Standard Insulin by the Rabbit and Mouse Methods of Assay, League Nat. Quat. Bull. **5:** 651, 1936.
6. Hell, C.: Ueber eine einfache Bildung von Stilben Mono-4-methoxystilben und α-Methylstilben, Ber. **37:** 453-458, 1904.
7. Scott, D. A., and Fisher, A. M.: The Insulin and Zinc Content of Normal and Diabetic Pancreas, J. Clin. Invest. **17:** 725-728, 1938.
8. U. S. Pharmacopea, ed. 12, Mack Printing Co., p. 233.
9. Young, D. M., and Lewis, A. H.: Detection of Hypoglycemic Reactions in the Mouse Assay for Insulin, Science **105:** 368-369, 1947.
10. Haist, R. E.: Factors Affecting the Insulin Content of the Pancreas, Physiol. Rev. **24:** 409-444, 1944.
11. Litchfield, J. T., and Wilcoxson, F., Jr.: A Simplified Method of Evaluating Dose Effect Experiments, J. Pharmacol. & Exper. Therap. **96:** 99-113, 1949.
12. Hoffman, W. S.: A Rapid Photoelectric Method for the Determination of Glucose in Blood and Urine, J. Biol. Chem. **120:** 51-55, 1937.
13. Barrnett, R. J., and Seligman, A. M.: Histochemical Demonstration of Sulfhydryl and Disulfide Groups of Protein, J. Nat. Cancer Inst. **14:** 769-803, 1954.
14. Barrnett, R. J., Marshall, R. B., and Seligman, A. M.: The Histochemical Demonstration of Sulfhydryl and Disulfide Groups in the Islets of Langerhans, Endocrinology.

THE SERUM VITAMIN B_{12} CONCENTRATION IN PERNICIOUS ANEMIA

ARNOLD A. LEAR, M.D.,* JOHN W. HARRIS, M.D.,** WILLIAM B. CASTLE, M.D.,*** AND ELEANOR M. FLEMING, A.B.
BOSTON, MASS.

THE microbiologic determination of vitamin B_{12} in body fluids, using *Euglena gracilis* var. bacillaris, was first described by Ross.[1] Earlier Hutner[2] had shown that *Euglena* exhibited a quantitative growth response to crystalline antipernicious anemia factor (vitamin B_{12}) when a synthetic medium was used. Harris,[3] Pitney and Beard,[4] and others have used this organism to assay vitamin B_{12} in serum and in urine. Since the publication by Harris[3] of preliminary data from this laboratory, subsequent modifications have been made in the method employed. The intent of this presentation is to report recent results of microbiologic assays of serum using this organism.

MATERIALS AND METHODS

Sterile serum samples were obtained from patients and stored at -20° C. until assayed. Assays were set up according to the method of Ross,[5] except that instead of a single dilution of each serum sample, serial dilutions were prepared for vitamin B_{12} estimation. Strict precautions were taken[6] in order to eliminate contamination and to insure vitamin B_{12}-free glassware. After eight days in the synthetic medium[7] containing added crystalline vitamin B_{12}, growth of the standard inoculum was measured turbidimetrically. Comparison of growth responses effected by serum samples with those produced by known amounts of crystalline vitamin B_{12} (USP Cyanocobalamin Reference Standard)† enabled estimation of the growth-promoting effect of serum for *Euglena*. Results are reported as vitamin B_{12} concentrations, assuming that the growth-promoting activity of serum is an index of its vitamin B_{12} content. Growth-supporting activity of native serum for *Euglena* is maximal when the diluted serum, after being added to the medium, is heated to 100° C. for 15 minutes. The total vitamin B_{12} concentration is thus determined. Values obtained prior to heating represent the so-called free vitamin B_{12} content of serum. The difference between free and total vitamin B_{12} levels has been considered to indicate the amount of vitamin B_{12} loosely bound to serum protein. The capacity of human serum to bind vitamin B_{12} in vitro was estimated by adding various amounts of crystalline vitamin B_{12} to serum and assaying the resultant samples before and after dilution and heating.

RESULTS

Serum Vitamin B_{12} Concentrations.—Assays of serum vitamin B_{12} content were done on normal subjects; on patients with pernicious and other nutritional macrocytic anemias; and on patients with other miscellaneous conditions, including a group with hepatic cirrhosis (Table I).

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.

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*Fellow in the Medical Sciences administered by the National Research Council for the Lilly Research Laboratories.

**Formerly Research Associate in Medicine, Harvard Medical School, and Research Fellow in Medicine of the American College of Physicians.

***Professor of Medicine, Harvard Medical School; Director, Thorndike Memorial Laboratory; Director, Second and Fourth Medical Services, Boston City Hospital.

†U.S.P. Cyanocobalamin Reference Standard was kindly supplied by Dr. Lloyd C. Miller.

TABLE I. SERUM VITAMIN B_{12} CONCENTRATIONS

GROUP	NUMBER OF SUBJECTS	TOTAL VITAMIN B_{12} LEVEL	
		RANGE	MEAN ± S.D.* ($\mu\mu$G/ML.)
Normal	20	292-856	532 ± 161
Pernicious anemia in relapse	33	0-85	39 ± 26
Pernicious anemia in remission	22	123-1,330	525 ± 337
Neurologic disease	14	195-760	439 ± 170
Achlorhydria	9	225-800	450 ± 234
Folic acid deficiency	12	148-557	307 ± 130
Hepatic cirrhosis	29	193-2,200	714 ± 534
Miscellaneous	40	115-1,029	- -

*S.D. = Standard deviation.

Normal Subjects.—Serum samples from twenty healthy normal persons 20 to 35 years of age yielded total vitamin B_{12} concentrations ranging from 292 to 856 $\mu\mu$g per milliliter with a mean concentration of 532. Free (unbound) vitamin B_{12} was found in all samples and ranged from 27 to 104 $\mu\mu$g per milliliter.

Patients With Pernicious and Other Nutritional Macrocytic Anemias.—Assays were done on 45 serum samples obtained before treatment from thirty-three patients with a well-established diagnosis of pernicious anemia. Included in this group were nine patients with combined system disease and pernicious anemia in hematologic relapse, as well as three patients with marked combined system disease and mild anemia who had been treated with oral preparations containing folic acid. In none of these samples was free vitamin B_{12} detected. Values for total serum vitamin B_{12} were observed ranging from 0 to 85 $\mu\mu$g per milliliter, with a mean of 39.

Twenty-two patients with pernicious anemia in remission who were receiving varied parenteral therapy had total vitamin B_{12} concentrations ranging from 123 to 1,330 $\mu\mu$g per milliliter with a mean of 525. Blood for these determinations was taken not less than four weeks after the last intramuscular dose of vitamin B_{12}.

Twelve patients with macrocytic anemia and megaloblastic bone marrows, all of whom responded to folic acid therapy, had levels ranging from 148 to 557 $\mu\mu$g per milliliter with a mean of 307.

Serum samples were also assayed from fourteen patients with neurologic evidence of posterolateral column disease without typical pernicious anemia. Of this group, five patients had no free hydrochloric acid before or after histamine stimulation. The total serum vitamin B_{12} levels ranged from 195 to 760 $\mu\mu$g per milliliter with a mean of 439.

Nine patients with achlorhydria persisting after histamine administration, some of whom had mild anemia, had total serum vitamin B_{12} concentrations ranging from 225 to 800 $\mu\mu$g per milliliter with a mean of 450.

Three additional patients (not reported in Table I) with macrocytic anemia had total serum vitamin B_{12} concentrations of less than 100 $\mu\mu$g per milliliter. One was a woman with macrocytic anemia associated with malnutrition and gastrointestinal malignancy on whom no further studies were

feasible. One was a patient who had had a total gastrectomy for peptic ulcers at a time when free gastric hydrochloric acid was present. The first vitamin B_{12} level was obtained approximately two years postoperatively. This patient showed no hematopoietic response to daily oral doses of 5 μg of vitamin B_{12}, but subsequently responded to daily oral doses of a preparation containing an equivalent amount of vitamin B_{12} plus intrinsic factor concentrate.* The third patient had had a portion of his terminal ileum resected because of regional ileitis. His serum vitamin B_{12} level was found to be low during a relapse, and was therefore treated with vitamin B_{12}. He responded hematopoietically and has since required maintenance therapy with parenteral vitamin B_{12}.

Patients With Other Conditions.—Assays of thirty-three sera from twenty-nine patients with hepatic cirrhosis showed a very wide range of total serum vitamin B_{12} concentrations, from 193 to 2,200 $\mu\mu$g per milliliter. Five patients in this group had levels greater than 1,000 $\mu\mu$g per milliliter without having received vitamin B_{12} therapy. The mean total concentration, including these extraordinarily high values, was 714 $\mu\mu$g per milliliter; excluding values above 1,000 $\mu\mu$g per milliliter, the mean was 496 $\mu\mu$g per milliliter. The free vitamin B_{12} levels were highly variable, ranging from 0 to 413 $\mu\mu$g per milliliter.

Sera from a heterogeneous group consisting of forty patients with anemias associated with iron deficiency, malnutrition, myxedema, hemolytic syndromes, and various chronic diseases had total serum vitamin B_{12} levels from 115 to 1,029 $\mu\mu$g per milliliter.

TABLE II. EFFECT OF 0.3 N NAOH AND BOILING UPON *Euglena* GROWTH-SUPPORTING ACTIVITY OF SERUM

	NUMBER OF DETERMINATIONS	AVERAGE TOTAL VITAMIN B_{12} LEVEL		*Euglena* ACTIVITY DESTROYED (PER CENT)
		BEFORE ALKALI ($\mu\mu$G/ML.)	AFTER ALKALI ($\mu\mu$G/ML.)	
Saline with added vitamin B_{12}	4	157	0	100
Normal serum	4	424	258	39
Pernicious anemia serum with added vitamin B_{12}	4	205	137	34
High level serum	2	1,510	788	48

Unusually High Concentration Levels (Table II).—Since the five patients with hepatic cirrhosis who had vitamin B_{12} concentrations greater than 1,000 $\mu\mu$g per milliliter had received no antecedent parenteral vitamin B_{12} therapy, further observations were carried out in order to determine whether this growth-supporting activity was due to compounds unrelated to vitamin B_{12}. For these observations serum was alkalinized with 0.3 N NaOH to pH>11, and thereafter was heated to 100° C. for 45 minutes. Prior to its use in serial dilutions, the serum was neutralized with 0.3 N hydrochloric acid. Assays done on normal sera and on sera with unusually high vitamin B_{12} levels showed that as a result of such alkalinization and heating, 39 and 48 per cent, respectively, of the *Euglena* growth-supporting activity had been destroyed. When crystalline vitamin B_{12} in saline was similarly alkalinized and heated,

*Kindly supplied by Dr. Kenneth W. Thompson of Organon, Inc., Orange, N. J.

however, all *Euglena* growth-supporting activity had been destroyed. Crystalline vitamin B_{12} was added to serum from four patients with pernicious anemia in relapse. These mixtures were similarly treated and thereafter estimation of *Euglena* growth-supporting activity indicated that only 34 per cent of the total activity had been destroyed.

Growth Inhibition.—In the course of routine analyses sera from three patients were found which inhibited *Euglena* growth. Investigation disclosed that these patients had been receiving sulfadiazine or sulfisoxazole therapy. The total serum sulfadiazine levels[8] ranged between 18.2 and 22.2 mg. per cent. Addition of para-aminobenzoic acid (PABA) to the serum prior to assay destroyed the inhibitory effect (Table III). Subsequent studies performed with saline solutions of sulfisoxazole, PABA, and vitamin B_{12} confirmed these observations (Table IV). Sulfisoxazole in concentrations as low as 1.6 mg. per cent inhibited *Euglena* growth in the presence of vitamin B_{12}. Added PABA prevented this inhibition but neither pteroylglutamic acid (PGA) nor citrovorum factor had such a corrective effect upon the inhibitory action of sulfisoxazole. Normal serum to which sulfisoxazole was added also showed an inhibitory effect upon growth; however, addition of PABA corrected the inhibitory effect and *Euglena* growth was then proportional to the vitamin B_{12} content of the sample assayed.

TABLE III. STUDY OF INHIBITION OF *Euglena* GROWTH

VITAMIN B_{12} (250 μμG/ML.)	SULFISOXAZOLE (20 μG/ML.)	PABA (1.25 μG/ML.)	PGA (100 μG/ML.)	CITROVORUM FACTOR (100 μG/ML.)	CHARACTER OF *Euglena* GROWTH
+					Normal
+	+				Inhibited
+	+			+	Inhibited
+	+		+		Inhibited
+	+	+			Normal
+		+			Normal
+			+		Normal
+				+	Normal

Saline dilutions of the substances indicated by + were assayed in the manner described.

TABLE IV. STUDY OF INHIBITION OF *Euglena* GROWTH BY SERUM

PATIENTS' SERUM	ADDED VITAMIN B_{12} (200 μμG)	SERUM SULFA LEVEL (MG. PER CENT)	ADDED PABA (1.25 MG./ML.)	CHARACTER OF *Euglena* GROWTH	SERUM B_{12} LEVEL (FREE/TOTAL) (μμG/ML.)
M. C.		22		Inhibited	–
		22	+	Normal	12/220
A. P.		18		Inhibited	
		18	+	Normal	100/405
C. S.		20		Inhibited	
		20	+	Normal	0/10
	+	20		Inhibited	
	+	20	+	Normal	13/210

Each tube contained serum plus added vitamin B_{12} and/or PABA as indicated by +.

Serum Binding Capacity.—Studies were undertaken in order to compare the capacity of normal and of pernicious anemia sera to combine with vitamin B_{12} in vitro (i.e., the sum of naturally present and of added vitamin B_{12} found to be unavailable to *E. gracilis* unless the serum is heated). Serum

samples from patients with pernicious anemia in relapse were mixed with equal volumes of 0.85 per cent saline containing amounts of vitamin B_{12} ranging from 200 to 1,000 $\mu\mu$g per milliliter. The concentration of total vitamin B_{12} increased in proportion to the amount of vitamin B_{12} added, the variation noted being consistent with the reliability of the method. The amount of vitamin B_{12} bound by serum also increased to a maximum and beyond this point the excess appeared as free vitamin B_{12}. This pattern was observed in four assays executed in this manner. The total binding capacity above which excess vitamin B_{12} appeared unbound ranged from 460 to 600 $\mu\mu$g per milliliter for undiluted sera from patients with pernicious anemia.

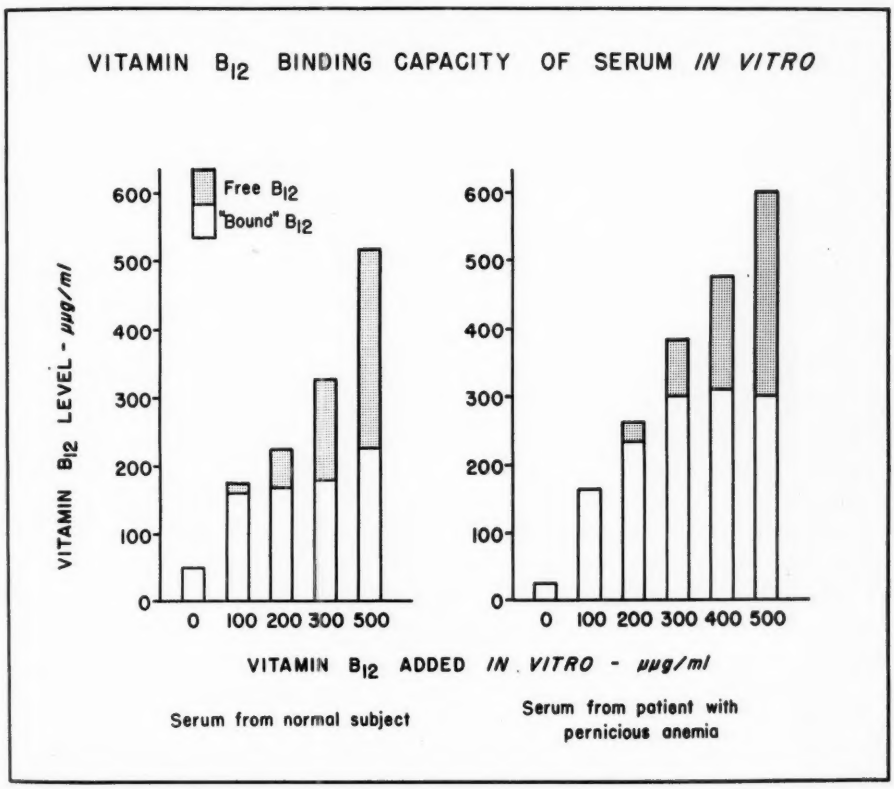

Fig. 1.—Comparison of binding capacity of serum from normal subject with that from patient with pernicious anemia.

The binding capacity of normal serum was studied in the same manner. The pattern of total vitamin B_{12} increase and of combining capacity maximum was similar to that demonstrated with pernicious anemia serum. The range of binding capacity of six normal sera was 450 to 660 $\mu\mu$g per milliliter (Fig. 1).

DISCUSSION

Serum Concentrations.—The absence of detectable free vitamin B_{12} and the low total vitamin B_{12} concentrations in the serum of patients with pernicious anemia in relapse confirm the clinical evidence as to the nature of the deficiency

in this disease. It appears reasonable to assume that the serum level reflects tissue concentration. Girdwood[9] reported that assays of tissues for vitamin B_{12} in patients with pernicious anemia in relapse showed no detectable level of vitamin B_{12}, whereas corresponding normal tissues contained measurable quantities. Therefore it is thought that the growth-supporting activity of serum for *E. gracilis* is most likely a function of the vitamin B_{12} content of serum or of closely related substances with hematopoietic activity.[5] Ford,[10] Lewis,[11] Robbins,[12] and Pfiffner[13] have shown that substances related to vitamin B_{12} derived from various sources all have cobalamin-like activity for microorganisms but not all influence hematopoiesis or animal growth. Such substances, however, have not yet been demonstrated in human serum.

The five patients with the unusually elevated vitamin B_{12} levels had one illness in common, hepatic cirrhosis. However, each had other diseases and differed clinically in terms of apparent severity of the liver disease, as well as the associated illnesses. None had received vitamin B_{12} therapy. Repeat estimations taken after several days again showed high serum levels in two patients. Attempts to destroy the vitamin B_{12} activity by alkalinization and heat succeeded in decreasing growth-supporting activity of the sera by 48 per cent (average of determinations). This was also true for normal serum and for serum of patients with pernicious anemia in relapse to which vitamin B_{12} had been added. These observations suggested that serum in some manner prevented complete degradation of its *Euglena* growth-supporting property, since vitamin B_{12} in saline so treated lost this property entirely.

Mollin and Ross[14] noted that 15 of 186 sera assayed by them had exceptionally high total vitamin B_{12} levels (1,000 to 4,000 $\mu\mu$g per milliliter) occurring principally as uncombined (free) activity. Included were sera from normal persons and from patients with pernicious anemia in relapse. They suggested that either the substance responsible for the high levels was hematopoietically inactive although possessing *Euglena* growth-supporting activity, or the extraordinarily high values were due to accidental contamination with vitamin B_{12}. That the exceptional values which we observed were due to compounds other than vitamin B_{12} with *Euglena* activity is possible but not proved. As most of the *Euglena* activity appeared in the bound form, however, it most likely represents vitamin B_{12} or a closely allied substance.[14] Hence it is conceivable that there was a true elevation of serum vitamin B_{12} due to an increase of the serum vitamin B_{12} binding property in these patients.* The characteristically elevated and abnormal globulins of such patients come to mind.

Growth Inhibition.—The inhibition of *Euglena* growth by sulfisoxazole is corrected by PABA. This indicates the dependence of this organism for synthesized PABA, inasmuch as PABA as such is not present in the synthetic medium. That PGA and citrovorum factor did not correct the sulfisoxazole inhibition indicates that for this organism PABA is concerned with other cell processes besides folic acid synthesis[15] such as nuclei acid and/or amino acid

*Since this manuscript was submitted for publication, Beard and associates (Blood 9:789-794, 1954) have described an increase in the vitamin B_{12} concentration and in the binding capacity of serum from patients with chronic myeloid leukemia.

synthesis. The observation that a sulfonamide derivative can inhibit growth if the serum sulfonamide concentration is sufficiently elevated demonstrates the need for considering this sulfonamide effect a possible explanation for the apparently depressed vitamin B_{12} levels found in patients receiving this therapy. The addition of PABA to the media abolishes this inhibitory effect. Ross[5] has stated that the serum concentrations of common antibiotics ordinarily attained in man do not inhibit growth.

Serum Binding Capacity.—The studies here reported indicate that serum contains a factor or factors which exert a binding effect on vitamin B_{12} and make it unavailable to support growth of *E. gracilis* until this effect is destroyed by heating. No difference in capacity to bind vitamin B_{12} was observed between sera from normal individuals and from patients with pernicious anemia; Pitney[16] reported similar results. Schilling[17] has shown by paper electrophoresis that vitamin B_{12} labeled with radioactive cobalt (Co^{60}) moves with albumin; and Pitney,[18] by microbiologic technique, has suggested that vitamin B_{12} binding is a specific property of the alpha globulin fractions of serum. Others[19-21] have shown a nonspecific binding effect by other protein-containing materials. Whether this binding property is a function of a specific component of serum serving as the vitamin B_{12}-transport vehicle as indicated by Pitney,[18] or whether this binding effect is nonspecific, is not firmly established. That a specific vitamin B_{12} binding or transport deficiency may exist has been suggested by Horrigan and Heinle[22] in a case report.

SUMMARY

The results of microbiologic assays of serum vitamin B_{12} levels using *E. gracilis* are reported. The mean total serum vitamin B_{12} level in twenty normal subjects was 532 $\mu\mu$g per milliliter; all had free vitamin B_{12} present. In thirty-three patients with pernicious anemia in relapse, it was 39 $\mu\mu$g per milliliter and no free vitamin B_{12} was detected. Twelve patients with megaloblastic anemia that subsequently responded to folic acid therapy had levels within the normal range and a mean of 307 $\mu\mu$g per milliliter.

Sulfonamide derivatives may be present in serum in concentrations which can inhibit *Euglena* growth; this inhibitory effect may be abolished by PABA added in vitro.

The capacity to bind vitamin B_{12}, thus rendering it unavailable for *Euglena* growth, is similar for serum from normal persons and from patients with pernicious anemia.

REFERENCES

1. Ross, G. I. M.: Vitamin B_{12} Assay in Body Fluids, Nature, London **166**: 270-271, 1950.
2. Hutner, S. H., Provasoli, L., Stokstad, E. L. R., Hoffman, C. E., Belt, M., Franklin, A. L., and Jukes, T. H.: Assay of Anti-Pernicious Anemia Factor With *Euglena*, Proc. Soc. Exper. Biol. & Med. **70**: 118-120, 1949.
3. Harris, J. W.: Vitamin B_{12} Levels in Pernicious Anemia, J. Clin. Invest. **31**: 637, 1952.
4. Pitney, W. R., and Beard, M. F.: Serum Vitamin B_{12} Concentrations in Pernicious Anemia, J. LAB. & CLIN. MED. **42**: 928, 1953.
5. Ross, G. I. M.: Vitamin B_{12} Assay in Body Fluid Using *Euglena gracilis*, J. Clin. Path. **5**: 250-256, 1952.
6. Robbins, W. J., Hervey, A., and Stebbins, M. E.: Studies on *Euglena* and Vitamin B_{12}, Bulletin of the Torrey Botanical Club **77**: 423-441, 1950.

7. Hutner, S. H., Provasoli, L., Schatz, A., and Haskins, C. P.: Some Approaches to the Study of the Role of Metals in the Metabolism of Microorganisms, Proc. Am. Phil. Soc. **94**: 152-170, 1950.
8. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537-550, 1939.
9. Girdwood, R. H.: The Occurrence of Growth Factors for Lactobacillus Leichmannii, Streptococcus Faecalis and Leuconostoc Citrovorum in the Tissues of Pernicious Anaemia Patients and Controls, Biochem. J. **52**: 58-63, 1952.
10. Ford, J. E., Kon, S. K., and Porter, J. W. G.: The Multiple Nature and Potency for Different Microorganisms of the Vitamin B_{12} Activity of Calf Rumen Contents and Faeces, Biochem. J. **50**: IX, 1951.
11. Lewis, U. J., Tappan, D. V., and Elvehjem, C. A.: A New and Biologically Different Form of Vitamin B_{12} J. Biol. Chem. **194**: 539-548, 1952.
12. Robbins, W. J., Hervey, A., and Stebbins, M. E.: Specificity of Vitamin B_{12} for *Euglena*, Nature, London **170**: 845, 1952.
13. Pfiffner, J. J., Dion, H. W., and Calkins, D. G.: Further Characterization of Pseudovitamin B_{12} Pigments, Fed. Proc. **11**: 269, 1952.
14. Mollin, D. L., and Ross, G. I. M.: The Vitamin B_{12} Concentration of Serum and Urine of Normals and of Patients With Megaloblastic Anaemias and Other Diseases, J. Clin. Path. **5**: 129-139, 1952.
15. Woods, D. D.: Biochemical Significance of the Competition Between Paraaminobenzoic Acid and the Sulfonamides, Ann. N. Y. Acad. Sc. **52**: 1199-1211, 1950.
16. Pitney, R., Beard, M., and Sanneman, E.: In Vitro Binding Capacity of Human Serum for Vitamin B_{12}, J. Clin. Invest. **32**: 595, 1953.
17. Schilling, R. F., and Deiss, W. P.: Intrinsic Factor Studies I. Paper Electrophoresis of Mixture of Gastric Juice and Radioactive Vitamin B_{12}, Proc. Soc. Exper. Biol. & Med. **83**: 506-509, 1953.
18. Pitney, W. R., Beard, M. F., and Van Loon, E. J.: Observations on the Bound Form of Vitamin B_{12} in Human Serum, J. Biol. Chem. **207**: 143-152, 1954.
19. Bird, O. D., and Hoevet, B.: The Vitamin B_{12}-Binding Power of Proteins, J. Biol. Chem. **190**: 181-189, 1951.
20. Ternberg, J. L., and Eakin, R. E.: Erythein and Apoerythein and Their Relation to the Anti-Pernicious Anemia Principle, J. Am. Chem. Soc. **71**: 3858, 1949.
21. Beerstecher, E., Jr., and Altgelt, S.: Apoerythein in Saliva, J. Biol. Chem. **189**: 31-34, 1951.
22. Horrigan, D. L., and Heinle, R. W.: Refractory Macrocytic Anemia With Defect in Vitamin B_{12} Binding and With Response to Normal Plasma, J. LAB. & CLIN. MED. **40**: 811, 1952.

NATURAL HISTORY OF KOREAN VIVAX MALARIA AFTER DELIBERATE INOCULATION OF HUMAN VOLUNTEERS

JOHN ARNOLD, M.D.,* ALF S. ALVING, M.D., ROBERT S. HOCKWALD, M.D.,**
CHARLES B. CLAYMAN, M.D.,** RAYMOND J. DERN, M.D.,***
AND ERNEST BEUTLER, M.D.****
CHICAGO, ILL.

ONE of the important medical problems encountered during the recent military activities on the Korean peninsula has been a very high incidence of vivax malaria. Prior to 1950 Korean malaria had attracted relatively little attention. Since 1950 a considerable body of information has developed relative to the natural history of the disease in man as well as to the response of this disease to drugs. Hankey and associates[3] reported on the relapse pattern in naturally acquired Korean malaria as it appeared in over 1,500 returning American military personnel, and Burnetti[1] reported on an outbreak of Korean malaria in thirty-four Camp Fire girls in California who had been infected by a carrier returned from Korea.

METHODS

It was our purpose in this study to observe the course of Korean vivax malaria produced by mosquito bites on human volunteers whom we could watch, under controlled circumstances, for an extended period. The strain of malaria was acquired by the United States Public Health Service at the Laboratory of Tropical Diseases in Milledgeville, Georgia, from a member of the United States Armed Forces returned from Korea, and was used to infect the mosquitoes used in this study. The six white male nonimmune subjects in the experiment were divided into two groups of three men each. On Nov. 13, 1951, each man in each group was bitten by ten infected mosquitoes in rotation with his two partners. Each mosquito was used to bite all three men in each group. These mosquitoes had been infected between Oct. 22 and Oct. 30, 1951. After biting the volunteers, the mosquitoes were dissected and the salivary glands were graded from 1 to 4 plus according to the number of sporozoites found in the glands. The grading system was on a logarithmic scale where 1+ was used for 1 to 9 sporozoites, 2+ for 10 to 99, 3+ for 100 to 999, and 4+ for more than 1,000 sporozoites seen in both glands. The average infection for each mosquito used was 3.6+ by this method of grading. Following inoculation these men were followed very closely with frequent examinations of the peripheral blood by thick smear. This was done daily until the first attack and then every other day for six weeks, after which time

From the Army Malaria Research Unit, Department of Medicine, University of Chicago, Chicago, Ill.

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*Formerly Major, MC, AUS.

**Formerly Captain, MC, USAR.

***Captain, MC, AUS, 9927-TSU-SGO.

****First Lieutenant, MC, AUS, 9927-TSU-SGO.

the smears were examined weekly until the volunteer again became patent. After each attack this sequence was repeated. Since Stateville is in a nonendemic zone, reinfection is not possible.

We were able to follow all six men up to and through at least one attack of malaria. One man, however, left prison four months after inoculation after having had only an early primary attack. One other man was discharged from prison after nearly one year, during which time he had one late primary attack. The other four men were followed to the apparent end of their malaria and three were followed about eighteen months beyond that point (June, 1954).

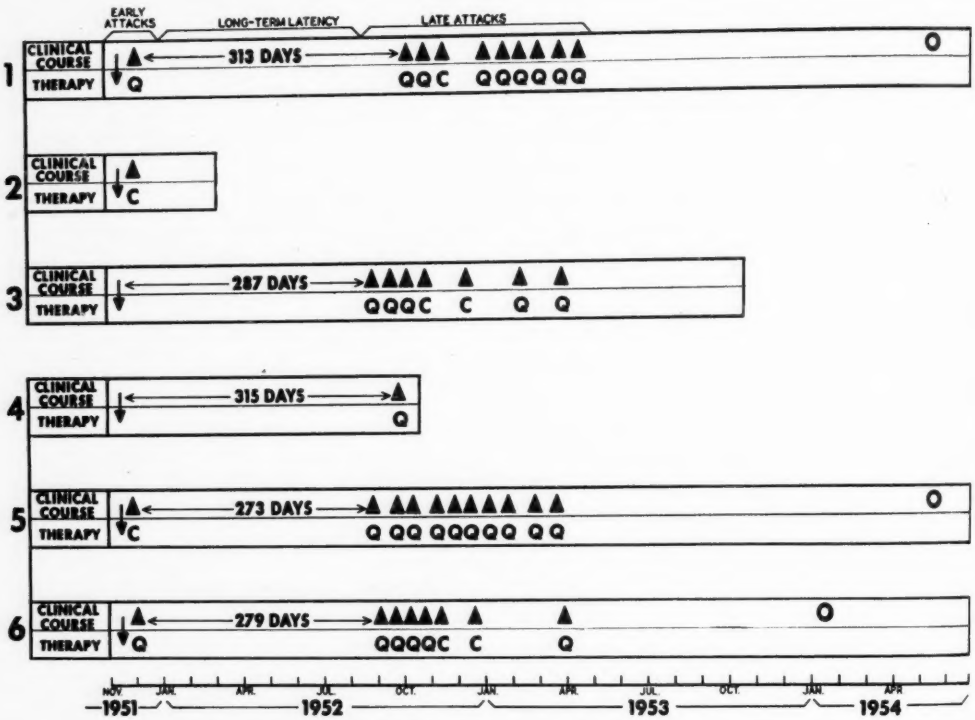

Fig. 1.

RESULTS

The most important observation to be drawn from the disease produced in these six volunteers was that the malaria of Korea is similar in relapse pattern to the malaria of other temperate zones. Temperate zone malarias usually have an early primary attack followed by a long latent interval, and then repeated late relapses. This pattern is apparent from Fig. 1. From this figure it can be seen that one man from each group of three failed to have a primary

attack while his two partners had a primary attack with patency beginning 18, 17, 16, and 16 days after infection. Whether or not a primary attack occurred seemed not to influence the length of the subsequent latent period which was amazingly uniform in length (273 to 315 days). Nor did the occurrence or lack of occurrence of a primary attack influence the length of time during which the patient had late relapses, or the frequency with which he had these late relapses. Since all men received a uniform and very heavy inoculation, the appearance or lack of appearance of an early primary attack seems not to be related to variation in the intensity of the sporozoite inocula.

Because our primary interest in these cases was directed at the natural history of this disease, we chose to treat each relapse with quinine or chloroquine. These drugs apparently do not disturb the tissue phases of vivax malaria. We also chose to treat each attack rapidly and vigorously in order to keep immunity at a minimum. It was hoped thereby to reduce the effect of immunity in limiting the course of the disease.

It is apparent that, after quinine treatment, relapses occurred with great rapidity and regularity. The average latent period from the end of quinine therapy to the next parasitemia was ten days. Chloroquine, by virtue of its long persistence in the body, appeared to abort one or two subsequent attacks, after which the previous periodicity would appear.

The behavior of these six heavily infected patients, in whom the exact date of infection was known, confirms the impression gathered from studies of malaria acquired naturally in the field, namely, that the disease terminates spontaneously before eighteen months. We were able to follow four cases from nine to eighteen months beyond this terminal period, and obtained no evidence of continuing activity. About three years after infection, 500 ml. of whole blood from each of three patients was subinoculated into three nonimmune volunteers by direct transfusion. The volunteers so inoculated remained negative for more than thirty days.

It would appear that clinical immunity to trophozoites is not a factor in the termination of the disease. It should also be pointed out that the initial infections were produced in November and that the clinical course of these patients was out of step by about six months with the seasonal incidence of naturally acquired Korean vivax malaria.

CONCLUSIONS

A strain of Korean vivax malaria was transferred by mosquitoes into six nonimmune white men and four of these subjects were followed to a point well beyond the apparent spontaneous termination of the disease. It appears from these cases that Korean vivax malaria is similar in its natural history to the other temperate zone malarias (Korteweg,[5] James and Shute,[4] Schüffner and associates,[6] and Coatney and colleagues[2]), and follows the observations already made on naturally acquired Korean vivax malaria (Hankey and associates[3] and Burnetti[1]). It is also apparent from these six cases that the season of the year has little direct effect on the relapse pattern once infection is produced, nor does the occurrence or lack of occurrence of an early primary at-

tack depend on a variation of the intensity of the sporozoite inoculum. From these six cases, as well as from other studies, it may be concluded that the disease terminates spontaneously at or about eighteen months after infection.

REFERENCES

1. Burnetti, R.: Outbreak of Malaria With Prolonged Incubation Period in California, a Non-endemic Area, Science **119**: 74-75, 1954.
2. Coatney, G. R., Cooper, W. C., Ruhe, D. S., Young, M. D., and Burgess, R. W.: Studies in Human Malaria. XVIII. The Life Pattern of Sporozoite-Induced St. Elizabeth Strain Vivax Malaria, Am. J. Hyg. **51**: 200-215, 1950.
3. Hankey, D. D., Jones, R., Jr., Coatney, G. R., Alving, Alf S., Coker, W. G., Garrison, P. I., and Donovan, W. G.: Korean Vivax Malaria. I. Natural History and Response to Chloroquine, Am. J. Trop. Med. & Hyg. **6**: 958-969, 1953.
4. James, S. P., and Shute, P. G.: Report on the First Results of Laboratory Work on Malaria in England, Geneva, 1926, League of Nations Health Organization.
5. Korteweg, P. C.: Klinische observaties over Malaria in den Winter van 1901-02, Herineringsbundel voor Prof. Rosensten, E. ijdo, Leiden, 1902, pp. 263-281.
6. Schüffner, W. A. P., Korteweg, P. C., and Swellengrebel, N. H.: Experimental Malaria With Protracted Incubation Period, Proc. Roy. Acad. Sc. Amsterdam **32**: 903-911, 1929.

PENICILLIN PROPHYLAXIS OF EPIDEMIC STREPTOCOCCAL INFECTIONS

I. The Epidemic and the Effects of Prophylaxis on the Clinical Manifestations of Acute Streptococcal and Nonstreptococcal Respiratory Infections

John R. Seal, Commander (MC) USN,* William J. Mogabgab, Lieutenant (MC) USNR, George J. Friou, Lieutenant (MC) USNR,** and James E. Banta, Lieutenant, junior grade (MC) USN***
Great Lakes, Ill.

INTRODUCTION

EPIDEMICS of streptococcal and other acute respiratory infections have continued to occur in the armed forces since the end of World War II.[1-3] Conforming to the pattern observed during the war years, these epidemics have been most severe in recruits and other populations in a training status.

High morbidity rates for uncomplicated streptococcal infections interrupt training schedules and necessitate a considerable expenditure of money and personnel for the care and treatment of these infections. Such purulent complications as sinusitis, otitis media, and peritonsillar abscess are frequently encountered. Outbreaks of rheumatic fever usually occur and acute glomerulonephritis may also be a sequel of epidemic streptococcal infections. These complications and sequelae aggravate the military medical problem because of longer hospitalization and occasional permanent disability. Adequate antibiotic therapy has reduced the incidence of complications and sequelae. To some extent it has also reduced the length of hospitalization necessary for uncomplicated streptococcal infections in those who have sought treatment. The failure of a large percentage of infected personnel to seek treatment for their illnesses, however, has prevented this advance in therapy from exerting any very obvious influence on the spread of streptococcal infections in military populations.[3, 4]

In the absence of other effective methods for control, investigations were begun into the use of antibiotics for the prevention of streptococcal infections in the spring of 1951.[4] Controlled studies were continued during the winter of 1951 to 1952 to obtain information on: (a) the minimum dose of

From the Naval Medical Research Unit No. 4, United States Naval Training Center, Great Lakes, Ill., and the Streptococcal Research Laboratory, United States Naval Training Center, Bainbridge, Md. Research Project NM 005 051.18, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

The opinions and assertions expressed herein are those of the authors and cannot be construed as reflecting the views of the Navy Department or of the Naval Service at large. The usage of commercially available products in connection with this study cannot be construed as indicating endorsement or approval of such products or as preference for one product over other similar products which are or may become commercially available.

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*Present address Preventive Medicine Division, Bureau of Medicine and Surgery, Navy Department, Washington 25, D. C.
**Present address Veterans Administration Hospital, West Haven, Conn.
***Present address School of Public Health and Hygiene, Johns Hopkins University, Baltimore, Md.

oral penicillin which would suppress epidemic streptococcal infections; and (b) the smallest dose of oral penicillin which would eradicate virulent strains of streptococci from the nose or throat of a majority of carriers of the organism. At the same time another group of workers on a military base were investigating the use of large doses of oral penicillin and of parenteral penicillin in epidemics and in carriers.[5]

The initial controlled studies indicated that either 100,000 units of oral penicillin G or 250 mg. of chlortetracycline (Aureomycin) once daily was effective in suppressing epidemic streptococcal infections by preventing men from acquiring group A streptococci during the period of prophylaxis.[4] Subsequent studies indicated that daily doses of penicillin ranging from 50,000 to 125,000 units were about equally effective in the prevention of streptococcal infections but were far less effective in eradication of the organisms from men already infected than were larger doses. Doses of either 125,000 units three times daily or 250,000 units twice daily for ten days were more effective in preventing men from acquiring epidemic strains in their nose or throat than were the smaller doses and were also effective in markedly reducing the carrier rates for group A streptococci.[6]

During the winter of 1952 to 1953, scarlet fever and other acute infectious diseases became epidemic at a naval training center and provided opportunity for investigation of differing dosage regimens of oral penicillin in entire regiments of Navy recruits. These studies were designed to provide additional information as to the possibilities of interrupting epidemics and of eradication of strains of streptococci causing the epidemic from large units of men.

In this paper, the epidemiologic and clinical aspects of respiratory infections occurring before and during prophylaxis are presented. These data provide the necessary background for evaluation of the results of oral penicillin prophylaxis. The prophylactic programs and their effects on admission rates for streptococcal infections and on carriers of beta hemolytic streptococci in the recruit populations in which they were used are presented in a subsequent paper.[7]

MATERIAL AND METHODS

Organization of the Naval Training Center and Populations.—The location of the base and organization of the Recruit Training Command have been partially described in a report on a prophylactic study carried out during the winter of 1951 to 1952.[2]

Male recruits in regular training were contained in three regiments, the first, second, and fourth. At the receiving unit men were organized into companies which varied in strength from sixty-four to seventy men during the study. Companies were assigned in rotation to the three regiments in so far as empty dormitories allowed. A majority of men in each new company were derived directly from civilian life. Men delayed on entry into training for preparatory training or by hospitalization while in the receiving unit were mixed with newly arrived recruits in the formation of companies. The normal processing time for a new recruit from time of arrival until entry into the regiment with his company was about two days. Naval reservists having had prior training duty were organized into companies scheduled for seven weeks of training. Regular Navy recruits went into companies scheduled for eleven weeks of training. Recruit barracks were

frame, two-storey buildings with a large open dormitory on each floor. At the beginning of the study one and one-half companies were assigned to each dormitory. Later, one company occupied each dormitory.

The company retained its location throughout training. Men losing five or more days of training through illness or other cause were reassigned to another company in the same week of training as was the man when he left his original company. The new company might be in a different regiment. During reassignment, most men spent twenty-four or more hours in barracks located in the fourth regimental area which were occupied by the reassignment unit. If the lost time was considerable, a longer period might be spent in these barracks while earlier training was reviewed. Recruits completing training were given two weeks' leave and then occupied the outgoing unit for a few days prior to their next duty assignment. This was located in close proximity to the receiving unit and men from these two units ate at the same mess hall.

The third regiment was partially occupied by WAVE recruits. Four barracks in the third regimental area were occupied by the recruit preparatory training unit. Men in this unit were mostly illiterate and were delayed from entering regular training up to nine weeks for instruction in reading and writing. Two other barracks in this area were occupied by companies of Naval Reserve recruits undergoing two weeks of training. Men in the third regimental area were strictly segregated from the WAVES although sharing the same mess hall and recreational facilities. They attended sick calls at the fourth regimental dispensary but had no other contact with this regiment.

Appreciable numbers of recruits were screened on arrival or during the first few weeks of training and placed under observation at the psychiatric unit. Many of the men who were under observation or who had been rejected for further training and were awaiting return to civilian life occupied barracks in the outgoing unit. Others were kept on the wards. Recruits committing disciplinary offenses were placed in the station brig in company of men from other commands.

As previously described, each recruit regiment was basically organized to be an independently functioning unit with its own medical department, dental department, stores, and mess hall. Because of personnel shortages, however, the second and fourth regiments shared the same mess hall after January. Opportunities for inter-regimental contacts also occurred in several special training activities, in various sports, and during recreation.

A second large, fairly homogenous population of enlisted personnel were the service school students. The majority of men in the schools were derived directly from graduates of recruit training of this and other training centers. Most schools were of fourteen weeks' duration and there was a steady flow of groups of men in and out of them at two-week intervals. Student barracks were similar in construction to those of recruits, which were adjacent to but separated from the first recruit regimental area. On the whole, the number of men per dormitory was equal to or greater than in recruit dormitories. During nonworking hours, students were less restricted in their activities than were recruits and enjoyed frequent liberty off the station.

A third large population of enlisted personnel upon which reasonably accurate strength data could be obtained was in the Administrative Command. This population was made up of heterogenous groups belonging to a number of departments such as Medical, Dental, Public Works, Commissary, and Security. Many worked in offices, others out-of-doors. Personnel of widely varied Navy experience and age were included. Unmarried men lived in barracks similar to those of recruits except that some were partitioned into rooms of various sizes. Again, dormitories were often more crowded than were recruit dormitories. Married personnel lived off the station. Rotation was slower in most of the groups making up this population than in either recruits or Service School students.

Various other small groups were also on the station. The Naval Academy Preparatory School students (about eighty men) occupied barracks in the Service School student living area but the men had little contact with other populations.

Recruits seeking treatment for illness or injury reported to their own regimental dispensary. Service School students and other groups reported at the main dispensary. Men with minor illnesses and injuries were hospitalized whenever possible in the small number of infirmary beds available. A vast majority, however, were admitted to a United States Naval Hospital which is also located on the station. Men from all populations came in intimate and prolonged contact on the infirmary and hospital wards. During convalescence, especially in the hospital, patients from different wards came in intimate contact at many work details and recreational facilities. This provided additional opportunity for interchange of infections in men just prior to their return to their command as most such patients were no longer receiving antibiotics. In general, patients hospitalized because of respiratory infections received four days' treatment with parenteral penicillin. In the spring months, many patients received 600,000 units of dibenzylethylenediamine dipenicillin G as the last dose of their treatment course. There was no specifically directed effort, however, to prevent cross infections in hospital or infirmary patients.

Clinical Procedures.—Arrangements were made with the senior medical officer of the Naval Training Center to have all except severely ill patients being admitted with a diagnosis of any type of acute respiratory infection brought to the laboratory en route to the hospital or infirmary. Also brought were patients with a diagnosis of any other infectious disease or complication of a respiratory infection, patients thought to have penicillin reactions, and patients with other diagnoses of some type of allergy. Patients in the latter two categories receiving outpatient treatment were also seen after prophylaxis was started.

The medical officers of the several dispensaries were liberal in their admission of patients. In addition to admitting patients with a temperature of 100° F. or greater required by medical department regulations, many men with afebrile minor illnesses resembling common colds were also admitted as can be seen in the data. Patients requiring admission were not treated prior to their transfer, although some had received antibiotics in an outpatient status at sick calls prior to the one at which admission was decided upon. Others had received antibiotics for unrelated reasons, such as dental treatment, within a day or so of admission.

Upon arrival at the laboratory each patient was examined by one of us who recorded the symptoms and physical findings and classified the illness according to predetermined criteria. This information was recorded on keysort cards as was information concerning the man's unit and location on the station; age; prior history of hospitalization, respiratory infections, and antibiotic treatment; tonsillectomy; and history of recent immunizations. The clinical diagnosis of the admitting physician was also recorded.

Symptoms such as chills, fever, prostration, weakness, fatigability of unusual degree, nausea, and vomiting were considered to be systemic and recorded under a single heading. The symptoms of myalgia, arthralgia, and headache were separately recorded and all symptoms were classified as being minor or major evidences of the illness. Careful questioning was done to establish the presence of sore throat and patients having dysphagia were distinguished.

Emphasis in the physical examination was on the presence of cutaneous rashes, abnormal findings in the nose or throat, the degree of tenderness of the anterior cervical lymph nodes, and the admission oral temperature. The throat was examined using a miner's-type headlight giving a bright white light. When exudate was seen, the throat swab was used to dislodge a part of it and establish its identity by demonstration of an inflammatory base.

A total leukocyte count was obtained on each patient during the examination. All patients admitted to the United States Naval Hospital with a diagnosis of an acute respiratory infection routinely received a roentgenogram of the chest upon admission with further roentgenograms being obtained if the diagnosis of pneumonia was suspected. These results were made available to us and were also entered on the keysort cards.

Bacteriologic Procedures.—The throat culture was taken with a sterile cotton swab under direct vision by the examining physician. The swab was immediately rolled on one side of a Petri dish containing 5 per cent sheep's blood agar (blood agar base BBL with sterile defibrinated sheep's blood added). A platinum loop was used to streak from the inoculated area to the balance of the media surface. Cuts in the agar perpendicular to the streaks were made to establish semianaerobic conditions for some colonies. Streaking was done within 30 minutes of obtaining the culture. The Petri dishes were then incubated at 37.5° C. until growth and hemolysis were adequate, usually 18 to 24 hours. Examination was then made for colonies showing typical beta hemolysis by the two technicians who read and recorded the results of all cultures. Dubious colonies were checked by gram-stained smears and subculture prior to being recorded as negative. Subcultures were made in a majority of instances from positive cultures to obtain isolated colonies. The number of hemolytic colonies was recorded as one plus if less than 10, two plus if more than 10 but not a pure culture, and three plus if a pure or very nearly pure culture. The type of colony was also recorded as being mucoid, matt, or glossy.

All colonies of beta hemolytic streptococci were serologically identified by the method of Swift, Wilson, and Lancefield.[8] Just prior to the preparation of the cellular extract, subcultures were made from the broth tubes to determine the purity of the culture and the penicillin sensitivity of the strain. Extracts were tested against antisera for Groups A, B, C, and G streptococci. Extracts giving precipitin reactions with Group A antisera were tested against antisera for Types 1 to 5, 8, 11 to 15, 17 to 19, 22 to 26, 28 to 33, 36 to 44, 46, and 47. These were examined for precipitin reactions after two hours' incubation at 37.5° C. and again after overnight refrigeration.

Penicillin sensitivity of streptococci was determined by the method of Frank, Wilcox, and Finland.[9] A loopful from the extract broth was streaked on the surface and stabbed in 5 per cent sheep's blood agar containing 0.04 unit of potassium penicillin G per milliliter. Each plate was examined after 24 and 48 hours' incubation along with a control plate prepared from the same broth. If typical beta hemolytic streptococci did not grow on the control plate or the broth was contaminated, the procedure was repeated from the original subculture plate. Each batch of media was checked with strains of streptococci which were not Group A and for which the sensitivity had been previously determined.

Classification of Illnesses.—For the purpose of recording admission rates for total acute respiratory infections and for streptococcal infections on a uniform basis before, during, and after courses of penicillin prophylaxis, the arbitrary exclusive classifications listed below were made on all cases with an admission diagnosis of acute respiratory infection when the results of the total leukocyte count, throat culture, and roentgenogram of the chest were available:

A. *Streptococcal infections*

1. All cases of scarlet fever.
2. All cases of acute respiratory infection with a positive throat culture for Group A streptococci and one of the following clinical syndromes:
   (a) exudative tonsillitis or pharyngitis; (b) dysphagia or very tender cervical lymph nodes and a leukocytosis of 12,000 or higher; (c) dysphagia and very tender cervical lymph nodes; (d) systemic symptoms and a leukocytosis of 12,000 or higher; (e) fever of 100° F. or more and a leukocytosis of 12,000 or higher; (f) leukocytosis of 16,000 or higher.

B. *Acute febrile, nonstreptococcal, respiratory infections*

1. Cases with a negative throat culture and either systemic symptoms or fever on admission.
2. Cases with a positive throat culture and either systemic symptoms or fever on admission and a total leukocyte count less than 12,000 cells per cubic millimeter.

C. *Other respiratory infections*

1. Cases with symptoms of more than 120 hours' duration.
2. Cases with exudative pharyngitis or tonsillitis and a negative throat culture.

3. Cases without systemic symptoms not qualifying for inclusion in categories A or B mentioned previously.
4. Cases with residual symptoms from a preceding respiratory infection.
5. Cases with roentgenographic finding of pneumonia.

D. *Erroneous diagnoses*
Diagnosis of an acute respiratory infection made by admitting physician but not concurred in by examiner.

Penicillin Administration.—Details of the program are presented elsewhere.[7] The fourth regiment of recruits was given 250,000 units of oral penicillin twice daily between February 7 and February 14, and again between April 5 and April 21. The second regiment was given 50,000 units of oral penicillin once daily between February 14 and February 28, and March 25 and April 11. The first regiment was also given 50,000 units between March 25 and April 11. Men with a prior history of hypersensitivity reactions to penicillin were excused. New recruit companies added to the regiment were given penicillin for whatever time remained of the schedule.

RESULTS

Epidemiologic Observations.—Following the conclusion of the prophylactic study in the spring of 1952,[2] scarlet fever occurred in increasing frequency. The prevalent type of streptococcus was found to have shifted from Type 12 to Type 19, only a few of which had been found during the earlier study.[10] Scarlet fever was epidemic from April through June, disappeared during the summer months, and began to recur in late September.

Brief inquiry into the situation was made by one of us (J. R. S.) in early November. In the preceding four weeks, admission rates for acute respiratory infections from the recruit population had increased from sixteen to twenty-eight per thousand per week. The diagnosis of acute tonsillitis, acute pharyngitis, or scarlet fever had been made in more than one-half of the admissions during this period and the incidence of exudative tonsillitis or pharyngitis was high among the few patients admitted during the inquiry. A majority of the patients with scarlet fever had been admitted from the fourth regiment, the regiment with highest admission rates. Nose and throat cultures were obtained from 167 recruits who had been on the station less than five days. Eight per cent had beta hemolytic streptococci in their nose or throat. One per cent had streptococci in both the nose and throat. Another 674 recruits in their first week of training (on station seven to ten days) were found to have a total incidence of 10 per cent positive cultures. Nose and throat cultures were both positive in 1 per cent. An additional 495 recruits in their ninth week of training were also cultured. A total of 19 per cent had positive cultures and in 5 per cent the nose and throat cultures were both positive. These differences served to establish that streptococcal infections were spreading among the recruits. In actuality the differences were more marked than is indicated by the per cent positive, as many of the new recruits had only a few glossy colonies in their positive cultures whereas old recruits more often had numerous large mucoid colonies resembling those that were identified as Type 19 streptococci. A majority of the strains isolated were not serologically identified.

In early December a small epidemiologic team headed by another of us (J. E. B.) conducted studies in the first regiment for about two weeks prior to the departure of a majority of recruits and other populations on holiday leave. A total of 389 patients complaining of respiratory infections were examined. Forty per cent could be reasonably classified as having streptococcal infections and 50 per cent had positive cultures for Group A streptococci of which three-fourths were Type 19. Type 1 was the only other prevalent type, occurring in 10 per cent of cultures positive for Group A streptococci. A leukocytosis of 12,000 or higher was present in 42 per cent of the 389 patients, with a majority of patients with exudative tonsillitis or pharyngitis and scarlet fever having a leukocytosis. Group A streptococci were recovered from only 10 per cent of patients having neither fever or leukocytosis and from 13 per cent of forty-six patients examined for complaints other than a respiratory infection.

On Jan. 4, 1953, immediately following the return of the majority of men from holiday leave, an outbreak of influenza occurred. This was first detected among convalescent rheumatic fever patients on one ward at the hospital. On December 28 and December 29, twelve of the approximately fifty patients on this ward developed a mild, febrile respiratory infection. Nasal washings and acute and convalescent blood sera were obtained from these twelve patients by the laboratory of the hospital and sent to Dr. Thomas G. Ward of the Johns Hopkins School of Hygiene and Public Health. An influenza virus, Type A′ similar to those isolated elsewhere in January, was isolated from eight of twelve washings studied and antibody rises occurred in the sera of three additional patients from whom viruses were not recovered. The influenza epidemic involved all populations, as can be seen in Fig. 1. Additional nasal washings were obtained from twelve selected patients from several populations in early January and yielded an A′ virus in seven instances. Acute and convalescent sera were also obtained from 100 selected admissions during the week ending January 10. A significant antibody rise to the FM-1 and to the prototype 1953 strain occurred in 65 per cent.

The epidemic terminated after the week ending January 17 with a marked decline in admission rates in the nonrecruit populations. Although cases of influenza probably continued to occur, this disease probably played little role in the continued high admission rates from recruit populations. Only nine (13 per cent) of sixty-nine pairs of sera collected from recruits admitted during the week of January 18 to January 25 exhibited significant antibody rises. This was almost identical with the percentage of significant antibody rises for influenza occurring in pairs of sera collected from patients with scarlet fever admitted during the same week.[11]

The number and classification of illnesses of patients admitted from the several major populations during and subsequent to the influenza epidemic are shown in Table I. The type distribution of streptococci found in men from these other populations was the same as in recruits.

No major outbreaks of any type of respiratory infection occurred in nonrecruit populations following the influenza epidemic, at least as reflected by

TABLE I. CLASSIFICATION OF ACUTE RESPIRATORY DISEASES ADMITTED FROM SEVERAL MAJOR POPULATIONS OF ENLISTED NAVAL PERSONNEL DURING PEAK OF INFLUENZA EPIDEMIC IN JANUARY, 1953, AND SUBSEQUENTLY (EXCLUDES ADMISSIONS DURING PENICILLIN PROPHYLAXIS AND FOR FOLLOWING WEEK IN RECRUITS)

CLASSIFICATION OF RESPIRATORY INFECTIONS		ADMISSIONS FOR RESPIRATORY INFECTIONS IN RELATION TO INFLUENZA EPIDEMIC*													
		DURING (JAN. 6 THROUGH 17)								POST. (JAN. 18 THROUGH MAY 30)					
		HOSPITAL CORPS SCHOOL		SERVICE SCHOOL STUDENTS		ADMIN. COMMAND		RECRUITS*		SERVICE SCHOOL STUDENTS		ADMIN. COMMAND		RECRUITS*	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Streptococcal infections	Scarlet fever	3	5	1	1	1	2	33	9	19	10	8	5	175	8
	Exudative pharyngitis	2	3	0	0	2	3	37	10	26	14	24	16	147	7
	Other	1	2	0	0	4	7	49	14	38	20	38	25	421	21
	Subtotal	6	9	1	1	7	12	119	34	83	45	70	46	743	37
Other respiratory infections	Exudative pharyngitis	0	0	4	5	0	0	5	1	11	6	5	3	28	1
	Systemic symptoms { present	54	85	61	82	44	73	139	40	55	30	48	32	320	15
	Systemic symptoms { absent	4	6	9	12	8	13	69	19	31	17	23	15	809	39
	Pneumonias	0	0	0	0	1	2	20	6	6	3	5	3	136	7
	Subtotal	58	91	74	99	53	88	233	66	103	55	81	54	1,293	63
Grand total		64	100	75	100	60	100	352	100	186	100	151	100	2,036	100
Average strength		1,398		3,318		3,130		7,600		3,153		3,080		6,305	

Apparent errors are due to rounding off of percentages.
*Except for pneumonias includes only those with untreated, acute, uncomplicated respiratory infections of less than 72 hours' duration.

admission rates. That a majority of the infections responsible for the continued elevation of admission rates among recruits were related to epidemic streptococcal infection in the recruit population will be shown in the following data of this and a subsequent paper.[7]

In addition to influenza, rubella occurred in the three recruit regiments in mildly epidemic form during the study. Sporadic in January and February, the incidence increased in March and April, then subsided again to sporadic levels as indicated by admission rates for this disease in Fig. 2.

Only cases are included where both the admitting physician and the examining physician agreed upon the diagnosis. The few cases in which they disagreed were usually substantiated to be scarlet fever. Thirty (14 per cent)

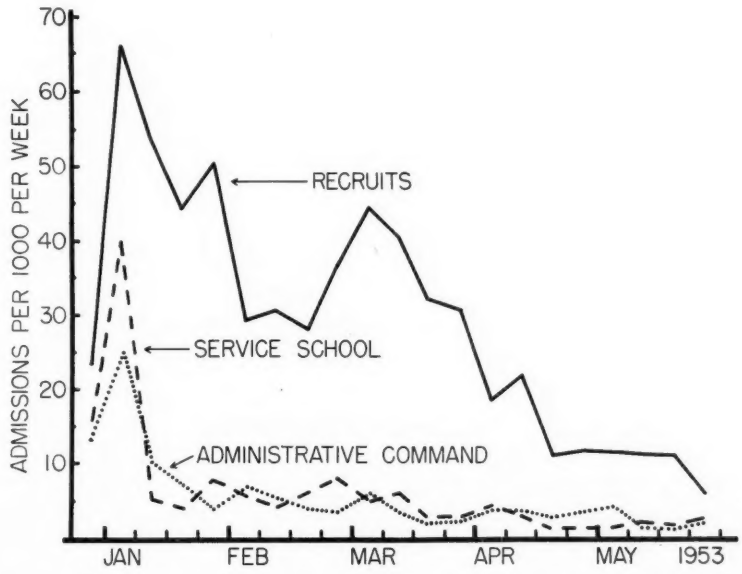

Fig. 1.—Admission rates for all acute respiratory diseases from three major populations during, and subsequent to, influenza A epidemic in January, 1953.

of a total of 218 patients examined during weeks when prophylaxis was not being given had positive throat cultures for Group A streptococci. None of the twenty-nine patients admitted from the fourth regiment during prophylaxis had positive cultures, but six (9 per cent) of the sixty-six patients admitted during administration of the 50,000 unit dose to the first and second regiments had positive cultures.

That not all cases with positive cultures were "carriers" was suggested by a review of cases with symptoms of less than forty-eight hours' duration. Thirteen (39 per cent) of the thirty-six patients with positive cultures had a total leukocyte count of 10,000 or higher whereas only forty-five (19 per cent) of the 234 patients with negative cultures had this degree of leukocytosis. If the percentage having a total leukocyte count of 12,000 or higher is compared, then eight (22 per cent) and twenty-four (10 per cent), respectively, had a

TABLE II. CLASSIFICATION OF 3,783 PATIENTS ADMITTED FROM THREE NAVY RECRUIT REGIMENTS WITH A DIAGNOSIS OF ACUTE RESPIRATORY INFECTION DURING WEEKS OF STUDY WHEN PENICILLIN PROPHYLAXIS WAS AND WAS NOT BEING GIVEN TO THE REGIMENTS

CLASSIFICATION OF RESPIRATORY INFECTIONS (EXCLUSIVE CATEGORIES)		FIRST REGIMENT 50,000 UNITS DAILY				SECOND REGIMENT 50,000 UNITS DAILY				FOURTH REGIMENT 500,000 UNITS DAILY				TOTAL PATIENTS IN WEEKS PROPHYLAXIS	
		22 WK. OFF		3 WK. ON		20 WK. OFF		5 WK. ON		20 WK. OFF		5 WK. ON			
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	OFF	ON
Acute streptococcal infections	Scarlet fever	104	8	0	0	64	6	4	1	53	6	0	0	221	4
	Exudative pharyngitis	72	5	4	3	58	6	4	1	65	7	1	1	195	9
	Systemic Symptoms present	165	12	7	5	122	12	14	5	125	14	0	0	412	21
	Systemic Symptoms absent	77	6	2	1	57	6	6	2	51	6	1	1	185	9
	Subtotal	418	31	13	9	301	30	28	9	294	33	2	2	1,013	43
Other acute febrile respiratory infections	Systemic Symptoms present	392	29	61	41	319	32	108	36	274	31	47	35	985	216
	Systemic Symptoms absent	265	20	43	30	190	19	76	26	165	19	46	35	620	165
	Subtotal	657	49	104	71	509	51	184	62	439	50	93	70	1,605	381
Miscellaneous respiratory infections*	Exudative pharyngitis	15	1	1	1	13	1	4	1	9	1	1	1	37	6
	Systemic Symptoms present	34	3	1	1	30	3	12	4	17	2	4	3	81	17
	Systemic Symptoms absent	170	13	22	15	125	13	60	20	90	11	30	21	385	112
	Residual or chronic	29	2	1	1	5	1	4	1	20	2	2	2	54	7
	Complicated	11	1	3	2	12	1	5	2	10	1	1	1	33	9
	Subtotal	259	20	28	20	185	19	85	29	146	17	38	28	590	151
	Total	1,334	100	145	100	995	100	297	100	879	100	133	100	3,208	575

Apparent errors are due to rounding off of percentages.

*Exudative pharyngitis with negative cultures, combined respiratory infection, and immunization reaction or purulent complications, afebrile respiratory infections without systemic symptoms not classifiable as streptococcal infections, those with a duration of symptoms exceeding 120 hours, and those with chronic symptoms not believed to have an acute respiratory infection.

leukocytosis. There was no correlation of either symptoms or height of the fever and a positive culture, although a correlation between these manifestations of rubella existed.

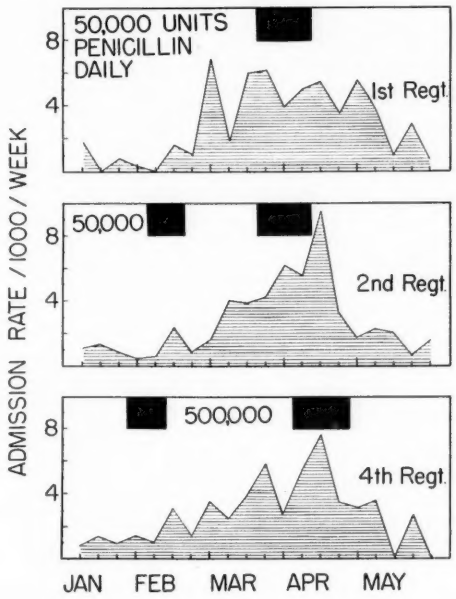

Fig. 2.—Admission rates for rubella from three regiments of male Navy recruits during oral penicillin prophylaxis of streptococcal infection studies, 1953.

CLINICAL OBSERVATIONS

Total Patients From the Three Recruit Regiments.—During the twenty-five weeks of study between January and June, 1953, a total of 3,862 patients were examined who had been admitted at their regimental dispensary with a diagnosis of some sort of acute respiratory infection. The recorded data on seventy-nine patients was too incomplete to allow classification. The final classification of the remaining 3,783 patients is given in Table II according to whether the admission occurred when prophylaxis was or was not being administered to the men in the regiment.

At examination the vast majority of patients (83 per cent while not receiving penicillin and 94 per cent while receiving penicillin prophylaxis) had symptoms referable to the respiratory tract but lacked any objective clinical features which were distinctive. Some of these were streptococcal infections according to the criteria used for classification, others were probably of streptococcal etiology but did not meet the criteria for being so classified. As was seen in Table I, only a small percentage of the total were admitted during the influenza epidemic in early January. Although influenza probably continued to occur in the recruit population and may have occurred as unrecognized outbreaks later in the study, the total numbers of cases were probably not large. The bulk of patients having negative throat cultures and of those with positive throat cultures lacking other evidences of streptococcal

infection fit the category of what has been referred to as "common respiratory disease of new recruits"[12] or as "ARD" (acute undifferentiated respiratory disease).[13]

One hundred fifty-six patients admitted during weeks in which prophylaxis was not being given had pneumonia reported on their admission x-ray film of the chest at the Naval Hospital. An additional thirty-eight with pneumonia were admitted during weeks in which prophylaxis was administered. A total of 209 other patients had either received an immunization within twenty-four hours of admission or had primary smallpox vaccination or some other immunization reaction at the time of admission. These patients,

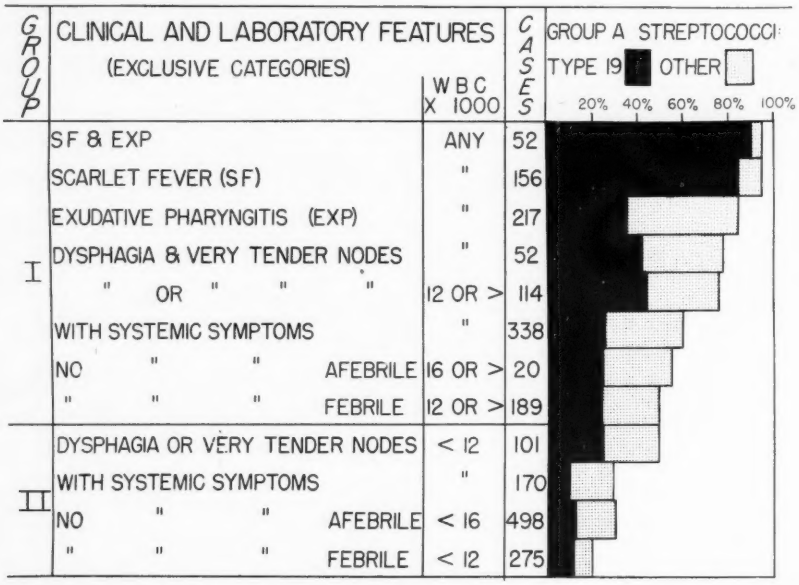

Fig. 3.—Relation of positive throat cultures for Group A streptococci to various clinical and laboratory features of acute respiratory infection in Navy recruits. Cases in Group I with positive culture classed as streptococcal infection, those in Group II not so classed.

in addition to all with complications or with a duration of symptoms of seventy-two hours or more, and all known or suspected of having had recent antibiotic treatment, were deleted to arrive at the numbers of patients with untreated acute, nonpneumonic, uncomplicated respiratory infections. Of the total of 2,735 which remained, 2,401 had been admitted during weeks of no prophylaxis and 334 during weeks of prophylaxis.

Clinical Characteristics of Untreated, Acute, Uncomplicated, Nonpneumonic Respiratory Infections in Patients Admitted Before and After Penicillin Prophylaxis and the Relation of These Characteristics to a Positive Throat Culture for Beta Hemolytic Streptococci.—From the total of 2,401 patients in this category, an additional 169 patients admitted during the first week following the several courses of penicillin in the three regiments were deleted. This was done to allow consideration of patients in regard to the results of throat cultures.

An analysis of the classification and clinical syndromes of the 2,232 patients remaining is presented in Table III. The percentage of patients having positive throat cultures for Group A streptococci is seen in Fig. 3.

TABLE III. POSITIVE THROAT CULTURES FOR BETA HEMOLYTIC STREPTOCOCCI FROM 2,232 NAVY RECRUIT PATIENTS ADMITTED WITH UNTREATED ACUTE, UNCOMPLICATED, RESPIRATORY INFECTIONS IN RELATION TO CLINICAL FEATURES OF ILLNESS (ADMISSIONS DURING PENICILLIN PROPHYLAXIS AND WEEK FOLLOWING EXCLUDED)

GROUP	CLINICAL FEATURES OF ACUTE RESPIRATORY INFECTIONS (EXCLUSIVE CATEGORIES)	NUMBER CASES	CULTURE FOR BETA HEMOLYTIC STREPTOCOCCI					
			POSITIVE					
			GROUP A				NOT GROUP A	NEGATIVE
			TOTAL	SEROLOGIC TYPE				
				19	OTHER*	UNTYPED†		
I	Scarlet fever and exudative pharyngitis	52	49	47	2	0		3
	Scarlet fever	156	147	132	10	5		9
	Exudative pharyngitis	217	184	80	91	13	6	27
	Dysphagia or very tender nodes, WBC 12,000 or >	114	90	48	34	8	2	22
	Dysphagia and very tender nodes	52	40	23	14	3		12
	Systemic symptoms, WBC 12,000 or >	388	236	103	122	11	4	148
	No systemic symptoms or fever, WBC 16,000 or >	20	11	5	6	0	2	7
	No systemic symptoms, febrile, WBC 12,000 or >	189	93	46	40	7	5	91
II	Dysphagia or very tender nodes, WBC < 12,000	101	50	26	21	3	3	48
	Systemic symptoms, WBC < 12,000	170	53	23	24	6	3	114
	No systemic symptoms or fever, WBC < 16,000	498	148	55	86	7	7	343
	No systemic symptoms, febrile, WBC < 12,000	275	57	34	17	6	10	206
	Total	2,232	1,158	622	467	69	42	1,032

Group I. In addition to all cases, scarlet fever all cases with features and positive culture for group A streptococci classified as streptococcal infections.

Group II. Not classified as streptococcal infection if culture positive.

*Types 1, 3, and 12 mostly, also Types 2, 5, 6, 11, 13, 18, 23, 28, 43.

†Did not type in available sera.

A problem that enters into this treatment of the data is the accuracy of the methods for culture in recovering streptococci present in the throat. It was not feasible to obtain multiple sequential or simultaneous cultures. Since 94 per cent of patients with scarlet fever or with exudative tonsillitis-pharyngitis and a marked leukocytosis (Table IV) had positive cultures, the methods apparently were highly accurate in streptococcal infections. Where the population of streptococci in the throat was less numerous, as was probably true in many chronic carriers, the methods were possibly less accurate, although there is no way of estimating to what degree.

The total leukocyte count, dysphagia, and very tender anterior cervical lymph nodes were afforded considerable weight in the criteria for recognition of streptococcal infections where neither scarlet fever nor exudative tonsillitis-pharyngitis was present. The analyses made in Table IV would seem to justify this.

TABLE IV. RELATION OF LEUKOCYTOSIS TO RECOVERY OF GROUP A STREPTOCOCCI IN THROAT CULTURE FROM NAVY RECRUIT PATIENTS WITH SCARLET FEVER, EXUDATIVE TONSILLITIS-PHARYNGITIS, OR ACUTE UNDIFFERENTIATED RESPIRATORY INFECTIONS

WHITE BLOOD CELL COUNT (× 1,000)	SCARLET FEVER*			EXUDATIVE PHARYNGITIS			UNDIFFERENTIATED RESPIRATORY INFECTIONS DYSPHAGIA AND/OR TENDER CERVICAL NODES					
							PRESENT			ABSENT		
		CULTURE POSITIVE			CULTURE POSITIVE			CULTURE POSITIVE			CULTURE POSITIVE	
	TOTAL CASES	NO.	PER CENT	TOTAL CASES	NO.	PER CENT	TOTAL CASES	NO.	PER CENT	TOTAL CASES	NO.	PER CENT
16 or >	60	57	95.0	84	79	94.0	83	67	80.7	252	168	66.7
12 to 15.9	43	40	93.0	69	58	84.1	70	57	81.4	383	191	49.9
10 to 11.9	36	34	94.4	38	25	65.8	61	32	52.4	318	110	34.6
< 10.0	17	16	94.1	26	22	84.6	53	24	45.3	587	129	22.0
Total	156	147	94.2	217	184	84.8	267	180	67.4	1,540	598	38.6

*Cases also having exudative tonsillitis-pharyngitis excluded.

Since nearly all patients had positive cultures, there was little relationship between the total leukocyte count and the chance of Group A streptococci being found in the throat culture in patients with scarlet fever. It was of interest that in 11 per cent of these patients the admission total leukocyte count was less than 10,000, and in 34 per cent, less than 12,000, cells. The latter count had been accepted as the minimal count for classifying patients as having a definite leukocytosis.

In patients with exudative tonsillitis-pharyngitis there was a definite relationship between the leukocyte count and the chance of the throat culture being positive. Ninety per cent of patients with total leukocyte counts of 12,000 or higher had positive cultures, whereas only 73 per cent of patients with lower counts had positive cultures.

The total leukocyte count was of more importance in patients lacking the previously mentioned features of streptococcal infection. In these patients there was a direct correlation between the degree of leukocytosis and the recovery of Group A streptococci on throat culture under the conditions which prevailed in this recruit population during the period of study. If dysphagia or very tender anterior cervical lymph nodes were present in addition to a leukocytosis of 12,000 or higher, streptococci were recovered with just slightly less frequency than in cases of exudative pharyngitis with leukocytosis. Even in the absence of leukocytosis, Group A streptococci were recovered from the throats of nearly one-half of the patients with one or both of these findings. When these findings were absent, however, only the presence of a marked leukocytosis gave a better than even chance of streptococci being recovered in the throat culture. There was a sharp break in the frequency with which patients having total leukocyte counts below 16,000 also had positive throat cultures. This decreased to where only one-third as many patients with counts below 10,000 yielded positive cultures as had patients with counts of 16,000 or more. The difference in frequency of positive cultures in patients with total leukocyte counts between 10,000 and

12,000 and in those with counts below 10,000 is an indication that the criteria adopted excluded some patients with streptococcal infections from being classified as such.

A total of 862 patients (75 per cent of those with positive throat cultures for Group A streptococci) with untreated, acute, uncomplicated respiratory infections met the criteria for classification as streptococcal infections. These are summarized in Table V.

TABLE V. CLINICAL AND LABORATORY FEATURES IN PATIENTS ADMITTED DURING PERIODS WHEN PENICILLIN PROPHYLAXIS WAS NOT BEING ADMINISTERED AND WHO WERE CLASSIFIED AS HAVING STREPTOCOCCAL INFECTIONS

CLINICAL FEATURES OF ACUTE RESPIRATORY INFECTIONS (EXCLUSIVE CATEGORIES)	PATIENTS	
	NUMBER	PER CENT
Systemic symptoms, WBC 12,000 or >	236	27.4
Scarlet fever	208	24.1
Exudative pharyngitis	184	21.4
No systemic symptoms, febrile, WBC 12,000 or >	93	10.9
Dysphagia or very tender nodes, WBC 12,000 or >	90	10.4
Dysphagia and very tender nodes	40	4.6
No systemic symptoms or fever, WBC 16,000 or >	11	1.3
Total	862	100.0

TABLE VI. POSITIVE THROAT CULTURES FOR GROUP A STREPTOCOCCI FROM 334 NAVY RECRUITS ADMITTED WITH UNTREATED ACUTE, UNCOMPLICATED, RESPIRATORY INFECTIONS DURING ORAL PENICILLIN PROPHYLAXIS IN RELATION TO CLINICAL FEATURES OF ILLNESS

GROUP	CLINICAL FEATURES OF ACUTE RESPIRATORY INFECTIONS (EXCLUSIVE CATEGORIES)	NUMBER CASES	POSITIVE CULTURE FOR GROUP A STREPTOCOCCI	
			TOTAL	TYPE 19
I	Scarlet fever	4	4	4
	Exudative pharyngitis	7	6	2
	Dysphagia or very tender nodes, WBC 12,000 or >	10	1	1
	Dysphagia and very tender nodes	3	2	0
	Systemic symptoms, WBC 12,000 or >	52	11	2
	No systemic symptoms or fever, WBC 16,000 or >	5	1	1
	No systemic symptoms, febrile, WBC 12,000 or >	32	3	2
II	Dysphagia or very tender nodes, WBC < 12,000	18	4	1
	Systemic symptoms, WBC < 12,000	85	6	3
	No systemic symptoms or fever, WBC < 16,000	49	5	3
	No systemic symptoms, febrile, WBC < 12,000	69	5	2
	Total	334	48	21

Group I. Classified streptococcal infection if culture positive for Group A streptococci.
Group II. Not classified streptococcal infection in event positive culture.

Acute, Uncomplicated, Nonpneumonic Respiratory Infections During Penicillin Prophylaxis.—The 334 patients who qualified for this classification and who were admitted from the three regiments during the weeks in which prophylaxis was being administered are summarized in Table VI. Nearly all of the patients with positive throat cultures were admitted from the first and second regiments during prophylaxis with 50,000 units of penicillin daily. Only four patients with positive cultures were admitted from the fourth regiment in the five weeks of prophylaxis with 500,000 units of penicillin daily. One of these had an exudative tonsillitis. Another qualified for classification as having a streptococcal infection while the remaining two had systemic symptoms and a total leukocyte count of less than 12,000.

Referring to Table III, a total of 1,188 (53 per cent of all patients admitted before or after prophylaxis) had clinical or laboratory findings which could lead to their classification as a streptococcal infection if a Group A streptococcus was recovered from their throat. The classification of streptococcal infection was assigned to 73 per cent of the 1,188. Only 115 (34 per cent) of the 334 patients admitted during prophylaxis had these clinical and laboratory findings. In only 24 per cent of the 115 were Group A streptococci isolated and a classification of streptococcal infection assigned. This suggested that in some cases which actually were streptococcal infections, the population of streptococci in the throat was so reduced by penicillin as to be missed by the single throat culture.

Relation of the Degree of Positivity of the Throat Culture to the Serologic Classification of Streptococci and the Incidence of Certain Clinical and Laboratory Findings in Patients.—A relatively high percentage of the streptococci isolated during the study formed many large mucoid or matt-mucoid colonies on the sheep's blood agar plates. These were usually identified as being Type 19. Other plates were seen to have smaller matt or glossy colonies. In order to determine the relationship between colonial growth and character and streptococcal infection, the amount of growth, colonial character, and serologic classification of all streptococci isolated from patients were examined.

Sixty-seven per cent of all Type 19 strains isolated were recorded as from plates containing a pure culture or predominant growth of matt or matt-mucoid colonies of beta hemolytic streptococci. In only 10 per cent had the colonies been recorded as appearing glossy with a majority of instances occurring in plates containing less than 10 colonies.

TABLE VII. DISTRIBUTION OF BETA HEMOLYTIC STREPTOCOCCI ISOLATED FROM PATIENTS WITH STRONGLY POSITIVE AND WEAKLY POSITIVE THROAT CULTURES ADMITTED DURING PERIODS WHEN PENICILLIN PROPHYLAXIS WAS NOT BEING ADMINISTERED ACCORDING TO CHARACTER OF COLONIAL GROWTH ON SHEEP'S BLOOD AGAR

CULTURE	NUMBER				PER CENT			
COLONIAL CHARACTER	MATT OR MUCOID		GLOSSY		MATT OR MUCOID		GLOSSY	
DEGREE POSITIVITY*	+++ OR ++	+	+++ OR ++	+	+++ OR ++	+	+++ OR ++	+
Group A Type 19	449	159	29	42	67	23	4	6
Type 1, 3, or 12	85	49	61	120	27	16	19	38
Other	26	9	22	24	32	11	27	30
Untyped	27	7	23	19	36	9	30	25
Total group A	587	224	135	205	51	19	12	18
Not group A	14	8	30	19	20	11	42	27

*+++Pure culture, ++Predominant growth, +Less than 10 colonies.

Only about one-third of all other Group A streptococci isolated were recorded as being from plates with many colonies appearing grossly similar to those formed by Type 19. Only about one-half of the other strains were recorded as being matt when viewed without the aid of magnification. The glossy appearance of Type 12 streptococcal colonies is probably one factor in this lowered percentage of matt colonies in strains of Group A other than Type 19.

Administration of penicillin prophylaxis not only reduced the number of patients with positive cultures but altered the distribution of strongly and weakly positive throat cultures in patients admitted during the periods of administration.

TABLE VIII. DEGREE OF POSITIVITY OF THROAT CULTURES FOR TYPE 19 AND OTHER BETA HEMOLYTIC STREPTOCOCCI FROM PATIENTS ADMITTED DURING PERIODS WHEN PENICILLIN PROPHYLAXIS WAS AND WAS NOT BEING ADMINISTERED

THROAT CULTURE		PATIENTS WITH POSITIVE THROAT CULTURES					
		BEFORE AND AFTER PROPHYLAXIS		DURING DAILY PROPHYLAXIS WITH			
				50,000 UNITS		500,000 UNITS	
SEROLOGIC CLASS OF STREPTOCOCCI	DEGREE OF POSIT.*	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Type 19	+++	205	30	9	31	0	
	++	286	43	7	24	0	
	+	183	27	13	45	1	-
Types 1, 3, and 12	+++	85	27	3	11	1	-
	++	120	38	10	37	0	
	+	110	35	14	52	3	-
Other types	+++	16	20	1	-	0	
	++	34	42	1	-	0	
	+	31	38	2	-	0	
Untyped	+++	18	24	1	-	0	
	++	28	37	2	-	1	-
	+	30	39	6	-	0	
Total	+++	324	28	14	20	1	-
Group A	++	468	41	20	29	1	-
	+	354	31	35	51	4	-
Not	+++	15	21	0		0	
Group A	++	18	25	0		0	
	+	38	54	12	100	1	-

*+++Pure culture, ++Predominant growth, +Less than 10 colonies.

Before and after prophylaxis, 69 per cent of the positive cultures of Group A streptococci were recorded as having beta hemolytic colonies in pure or predominant growth on the blood agar plate. During administration of 50,000 units of penicillin daily, a grossly equal amount of growth was observed in only 49 per cent of the plates recorded as being positive for beta hemolytic streptococci. Three-fourths of the Type 19 colonies were described as matt or matt-mucoid, while the same proportion of other Group A streptococcal colonies were described as being glossy. All colonies of strains other than Group A were glossy.

There was no marked difference in the proportion of cultures with many colonies or with few colonies between Type 19 and the other strains of streptococci. For convenience, those in which predominant growth or a pure culture were recorded are referred to as strongly positive, and those in which only a few colonies were recorded are referred to as weakly positive.

In an attempt to assess the differential importance of the degree of positivity of the throat culture, the incidence of various clinical and laboratory findings was compared between patients with positive cultures for Type 19, for other types, and for patients with negative cultures.

In examining these data it is important to recall that 75 per cent of patients with positive throat cultures for Group A streptococci met the criteria

TABLE IX. RELATION OF THE DEGREE OF POSITIVITY OF THE THROAT CULTURE FOR TYPE 19 AND OTHER GROUP A STREPTOCOCCI TO THE INCIDENCE OF CERTAIN CLINICAL AND LABORATORY FINDINGS IN PATIENTS* ADMITTED WITH RESPIRATORY INFECTIONS DURING PERIODS WHEN PENICILLIN PROPHYLAXIS WAS NOT BEING ADMINISTERED

ITEM		PER CENT OF PATIENTS WITH ITEM				
		19		OTHER		
THROAT CULTURE	TYPE DEGREE†	STRONG	WEAK	STRONG	WEAK	CULTURE NEGATIVE
Scarlet fever		27	15	2	2	1
Exudative pharyngitis		21	10	21	13	1
Admission oral Temperature ° F.	103	14	8	15	9	9
	102	33	30	36	29	24
	101 or >	58	51	62	49	44
	100	84	81	84	81	74
	99	96	95	95	96	91
Systemic symptoms		68	59	66	55	51
Myalgia		31	27	29	29	19
Arthralgia		6	7	6	4	6
Sore throat, total		88	75	88	77	68
dysphagia only		32	21	21	22	8
Tender cervical nodes, total		68	55	60	52	27
very tender nodes only		18	11	11	12	3
dysphagia and very tender nodes'		12	4	6	7	1
Admission total Leukocyte count	16,000	37	28	33	32	12
	14,000 or >	57	44	49	42	19
	12,000	68	62	68	58	34
	10,000	87	78	83	81	55
Number of patients		491	183	301	171	1,451

*Excludes patients with immunizations, complications, chronic or residual symptoms, and incomplete information on any item.

†Degree of positivity—strong indicates either pure culture or predominant growth, weak indicates less than ten colonies on sheep's blood agar plate.

for classification of a streptococcal infection as adopted for this study (Table V). Inclusion of patients admitted during the first week after the several periods of prophylaxis and of patients with a duration of symptoms up to 120 hours in the current analysis probably did not alter this percentage to any great degree.

One of the outstanding differences between patients with positive cultures for Type 19 and those with other Group A streptococci was the incidence of scarlet fever in relationship to Type 19. The few cases that occurred in patients not harboring Type 19 were caused by Types 1, 3, and 12. Although scarlet fever did not occur in as high a percentage of patients with weakly positive cultures, the incidence in such patients was still such that a weakly positive culture was of considerable importance. The figures in Table IX are misleading as to the true incidence of exudative pharyngitis in relationship to Type 19 streptococci. Approximately 25 per cent of patients with scarlet fever also had exudative tonsillitis-pharyngitis (Table III) and so the true incidence of exudative tonsillitis in those with Type 19 streptococci should be a few per cent higher. Despite this overlap, the two manifestations of streptococcal infection seem independent, with scarlet fever closely related to a particular type and exudative tonsillitis-pharyngitis unrelated to type.

In general, patients with strongly positive cultures departed further from patients with negative cultures in the incidence of the selected manifestations of respiratory infection than did patients with weakly positive cultures. This was seen mostly in relation to a fever of 103° F. or higher, systemic symptoms,

and the total incidence of sore throat and tender anterior cervical lymph nodes. The differences were more consistently observed in relation to Type 19 streptococci than in relation to the other types.

Major differences between the incidence of the selected clinical and laboratory findings in patients with negative cultures and in those with positive cultures occurred in only a few findings. These were scarlet fever, exudative tonsillitis-pharyngitis, myalgia, dysphagia, tender anterior cervical lymph nodes, and leukocytosis. While high fever tended to occur more frequently in those with strongly positive cultures, it was sufficiently frequent in patients with negative cultures to negate this difference as being clinically important. The same observation might be made about systemic symptoms such as chills or chilliness, feverishness, prostration, or headache. Nausea and vomiting were rarely encountered. Arthralgia had occurred in a minority of patients by the time of admission and did not appear to be influenced by the presence or absence of streptococci in the throat.

Thirty-four per cent of patients with negative throat cultures had total leukocyte counts of 12,000 or higher, and in 55 per cent the count was above 10,000. Despite this relatively high incidence of leukocytosis in nonstreptococcal infections among these men, the incidence was two to three times greater among men with positive throat cultures. (It is worth pointing out that the difference between patients with rubella and those with acute undifferentiated respiratory infections was about as much as between the latter and those with positive throat cultures.)

Incidence of Selected Clinical and Laboratory Findings in Patients With Negative Cultures for Beta Hemolytic Streptococci Admitted During Prophylaxis.—The remarkable effectiveness of the daily dose of 500,000 units of penicillin[7] suggested another approach in determining the probable accuracy of the cultural methods. Any effects of dose in altering the admission of patients with missed positive cultures should be evident in a lowered incidence of the more important clinical and laboratory features of streptococcal infection among patients with negative cultures. Accordingly, an analysis was made of patients with negative cultures admitted during the administration of prophylaxis with 50,000 units and with 500,000 units daily. Since the admission rates for all respiratory infections were much lower after the second period of prophylaxis than before the first and possibly reflected a seasonal change in the diseases, admissions during these periods before and after prophylaxis have been shown separately. Admissions during the interval between the first and second courses of penicillin in the second and fourth regiments have been included as after penicillin.

Outside of the elimination of scarlet fever, neither dose of penicillin appeared to have any marked effect on the incidence of these selected manifestations of acute respiratory infection. The percentage of patients with an admission temperature of 102° F. or over was slightly lower in both prophylactic groups without any increase in the percentage of patients without fever. In the group which received the larger dose there was also a slightly lower incidence of myalgia and dysphagia. These differences, while statistically

TABLE X. CLINICAL AND LABORATORY FINDINGS IN PATIENTS WITH NEGATIVE THROAT CULTURES FOR BETA HEMOLYTIC STREPTOCOCCI ADMITTED FOR ACUTE RESPIRATORY INFECTIONS BEFORE, DURING, AND AFTER ORAL PENICILLIN PROPHYLAXIS

RELATION TO PENICILLIN PROPHYLAXIS		BEFORE	DURING		AFTER
DAILY DOSE OF PENICILLIN		NONE	50,000 UNITS	500,000 UNITS	NONE
TOTAL NUMBER ADMITTED		684	318	105	766
Per cent of patients with	Admission oral temperature in ° F. 103	9	5	5	8
	Admission oral temperature in ° F. 102	24	19	15	24
	Admission oral temperature in ° F. 101 or >	41	39	34*	46
	Admission oral temperature in ° F. 100	68	76	70	78
	Admission oral temperature in ° F. 99	90	90	89	92
	Chills, prostration, etc.	46	50	41*	55
	Myalgia	19	14	8*	19
	Arthralgia	6	5	5	6
	Generalized headache	4	1	2	1
	Sore throat, total	61	80	72	73
	Sore throats and dysphagia	7	8	4	8
	Tender cervical nodes, total	24	26	24	29
	Very tender nodes	2	3	1	3
	Dysphagia and very tender nodes	1	1	0	1
	Admission total leukocyte count 16,000	11	11	11	13
	Admission total leukocyte count 14,000	17	22	19	21
	Admission total leukocyte count 12,000 or >	32	36	33	37
	Admission total leukocyte count 10,000	53	56	56	58
	Scarlet fever	1	0	0	1
	Exudative pharyngitis	2	2	1	1

*Statistically significant difference at P = < .05 level or greater.

significant, can be accorded no great clinical significance. It would seem fair to conclude that neither beta hemolytic streptococci or any other penicillin-sensitive organism had any important role in the illness of patients with negative throat cultures at admission.

Relation of Tonsils to Exudative Tonsillitis-Pharyngitis and to Scarlet Fever.—Fifty-one per cent of patients gave a history of having had a tonsillectomy. In men with a prior history of tonsillectomy, tonsillar remnants were frequently present and exudate was sometimes found on the remnants. Most often when the tonsillar fossae were virtually devoid of lymphoid tissue, any exudate seen was along the lateral margins of the posterior pharynx and was scanty in comparison to amounts seen in patients with tonsils.

As seen in Table XI there was a close relationship between the presence of tonsils and the finding of exudate which was independent of the type of streptococcus isolated from the throat. Considering only the three strains of streptococci which were prevalent and caused most infections, Types 1, 3, and 19, three to four times as many patients with positive cultures for these types and with tonsils had exudate visualized as did patients with these types and no tonsils. There was no apparent tendency for patients with tonsils to exhibit a higher percentage of positive cultures for either the prevalent or sporadic types of streptococci than was found in patients without tonsils. Neither was there any tendency for scarlet fever to occur more often in patients with tonsils.

Similar analyses of admissions during the period of prophylaxis were also made. The few cases with positive cultures that had exudate or scarlet

TABLE XI. RELATION OF TONSILS TO OCCURENCE OF EXUDATIVE TONSILLITIS-PHARYNGITIS (EXP) OR SCARLET FEVER (SF) IN PATIENTS* WITH POSITIVE AND NEGATIVE CULTURES FOR BETA HEMOLYTIC STREPTOCOCCI

STREPTOCOCCI IN THROAT CULTURE	TOTAL NO. CULTURED	TONSILS ABSENT			TONSILS PRESENT		
		NUMBER PATIENTS	PER CENT WITH		NUMBER PATIENTS	PER CENT WITH	
			EXP	SF		EXP	SF
Type 1	166	69	7	1	97	24	3
Type 3	156	78	8	1	78	28	0
Type 19	784	381	9	24	403	34	22
Other types†	75	36	3	0	39	26	3
Type 11‡	77	39	20	3	38	39	5
Untyped§	86	38	8	8	48	33	2
Total Group A	1,344	641	9	15	703	32	14
Not Group A	88	33	0	9	55	25	2
Negative	1,564	789	1‖	1	775	4‖	1
Total	2,996	1,463	4	7	1,533	18	7

*Excludes patients admitted during penicillin prophylaxis, with incomplete history of tonsillectomy, prolonged or chronic symptoms, and with only complications.

†Types 2(1), 5(9), 6(8), 12(52), 18(1), 23(1), 26(1), 28(1), 43(1).

‡May not be Type 11, obtained with only lot of antisera.

§Did not react with available antisera or were not tested.

‖Statistically significant difference ($P = < .01$).

fever did not allow any conclusion. Exudative pharyngitis was more common in those with tonsils regardless of the results of culture.

The data were further analyzed to learn if there was any relationship between tonsils and the degree of positivity of the throat culture. Considering only Type 19, there were 633 patients with complete information. Of 453 with strongly positive cultures, 207 or 46 per cent had at least the major portion of their tonsils removed while 49 per cent of 180 patients with weakly positive cultures had had a tonsillectomy. Of 278 with strongly positive cultures for types other than Type 19, 49 per cent, and of 158 with weakly positive cultures, 44 per cent, had had a tonsillectomy. These differences were not statistically significant.

DISCUSSION

Evaluation of the results of preventive measures aimed at one specific type of acute respiratory infection is at times a complex problem in populations which suffer multiple respiratory infections of varying etiology within a short time span. Most of the difficulty arises from a lack of simple methods for classifying respiratory infections into etiological entities and a considerable overlap in clinical phenomena in infections of diverse etiology. The experience of this Navy recruit population during the winter months was probably not an unusual one for any recruit population trained in areas where streptococcal infections become prevalent.

"Carrier" rates for Group A streptococci are usually found to be high at times when streptococcal infections are epidemic in a population. When epidemic or endemic respiratory infections of nonstreptococcal etiology occur in men carrying prevalent strains in their nose or throat at the same time epidemic streptococcal infections are occurring, the accurate differentiation of that proportion of illnesses actually due to streptococcal infection becomes especially difficult. In the preantibiotic era it was suggested that an elevation

of the antistreptolysin 0 titer was the preferred differential method.[14] Since antibiotics have come into general usage for the treatment of all febrile acute respiratory infections and may suppress the antibody response to streptococcal infection,[15] immunologic evidence is no longer reliable.

In the present study arbitrary criteria for the recognition of streptococcal infections had to be adopted in order to calculate admission rates for these infections. It naturally was of interest to attempt to assess the accuracy of the diagnosis as made by these criteria. One method for accomplishing this when immunologic data are lacking is to estimate the "carrier" rate from cases thought not to be of streptococcal etiology. In Table IV, 22 per cent of cases lacking such findings as scarlatinal rash, exudate in the pharynx, dysphagia, very tender cervical nodes, or a white blood cell count of 10,000 cells per cubic millimeter or higher had positive throat cultures for Group A streptococci. This is double the per cent of cases of rubella which were admitted from the same populations during the same weeks that had positive cultures, but for purposes of discussion the carrier rate might be assumed to lie somewhere between 11 and 22 per cent. If twenty per cent of the patients were carrying Group A streptococci in their throats which were unrelated to the respiratory infection causing admission, then presumably the excess of positives in any category in Table III or Fig. 3 would be due to actual streptococcal infection. This reasoning, if carried to its logical conclusion, would indicate that only 74 to 80 per cent of cases of scarlet fever were of streptococcal etiology. This is only slightly relieved if only those cases are considered from which Type 19 streptococci were isolated. As this is hardly reasonable, it is necessary to conclude that a considerable proportion of the cases with positive cultures excluded from being classed as streptococcal infections by the criteria were actually of this etiology. It has previously been pointed out in the epidemiologic background that there were suggestive evidences of dual or superimposed infection in those cases of rubella with positive cultures so even these cannot be accepted to represent true carriers in all instances. It may be that had the degree of positivity of the culture, the type of colonial growth, and the serologic type distribution been taken into account in the criteria, somewhat greater accuracy could have been achieved.

While scarlet fever is almost universally accepted as being caused by Group A streptococci, it is known that exudative tonsillitis-pharyngitis of nonstreptococcal etiology does occur. While large numbers of cases of exudative tonsillitis-pharyngitis have not been observed in Navy recruit populations in the absence of epidemic streptococcal infections, the problem of the examiner is differentiation of the single case. The Commission on Acute Respiratory Diseases described the main differential features of streptococcal and nonstreptococcal exudative tonsillitis and pharyngitis.[16] Streptococcal infections generally have a more acute onset with sore throat and systemic symptoms, higher fever, diffuse redness and edema of the mucous membranes of the pharynx, a discrete or confluent exudate usually on the tonsils, enlarged and tender cervical nodes, and a moderate leukocytosis. Nonstreptococcal

exudative tonsillitis and pharyngitis is generally milder, has a more gradual onset, the exudate is less extensive and may be confined to the posterior pharyngeal wall rather than the faucial tonsillar tissue, inflammation of the mucous membranes is less prominent, as is cervical lymphadenopathy, and the total leukocyte count is either normal or slightly elevated. They also pointed out that clinical differentiation, even when combined with the results of the throat culture, was not infallible. Under the conditions of the present study it would appear that the vast majority of patients with exudative lesions in the throat represented streptococcal infections. Although the total leukocyte count was of some value in differentiation, it afforded no exact separation and there was a considerable overlap in this feature between cases of nonstreptococcal and streptococcal exudative tonsillitis-pharyngitis. As had been pointed out by others,[17-20] it was also obvious in the results that the finding of exudate is dependent to a large degree on the proportion of the population who retain their tonsils.

The difference in type distribution of streptococci isolated from cases of exudative tonsillitis or pharyngitis and scarlet fever was of interest. The findings in scarlet fever were confirmed on reculture of these patients in the hospital. Few of the patients with scarlet fever admitted without exudate in the pharynx developed this finding under observation.[11] Thus, we have one classic manifestation of streptococcal infection caused almost entirely by a Type 19 streptococcus in which exudative tonsillitis or pharyngitis was not a prominent finding. In another group of cases it was a prominent feature and was unrelated to any particular type of streptococcus. In fact, the percentage of cases of scarlet fever with exudate and of nonscarlatinal streptococcal infections with exudate appeared to be about equal and suggested that exudative lesions in the pharynx are a characteristic of host reaction rather than the infecting streptococcus.

It was apparent that a majority of cases of streptococcal infection in the present series lacked either of the distinguishing features of scarlatinal rash or exudative lesions in the throat. Of the total of 2,232 cases observed, 1,807 lacked these findings. Positive cultures for Group A streptococci were obtained in 778 of the 1,807 cases. Sixty-two per cent of the positive cultures were isolated from cases with a total leukocyte count of 12,000 or more, whereas such cases comprised only 43 per cent of the 1,807. Even if one assumed the carrier rate to be 22 per cent (Table IV) only 182 of the 483 cases with positive cultures and a leukocytosis could be discounted as carriers of streptococci who were suffering from nonstreptococcal infections. Even when all cases with dysphagia or very tender cervical nodes are excluded, there still remains a high percentage of the total cases which must be considered streptococcal infections. These cases lack any distinctive finding which would set them apart from other nonstreptococcal respiratory infections save a positive culture. While the total leukocyte count may suggest the probable outcome of the throat culture, particularly if it is very high or is within normal limits, it probably would be of little help to an examiner in classification of a single case which lacked other distinctive features and does not appear to be as reliable as would be the results of a throat culture.

The criteria adopted were in many respects more conservative than those of Loge and Kilbourne.[21] During an epidemic of streptococcal infections in a military camp these workers employed the following criteria in the majority of cases: (1) fever greater than 100° F. on day of admission; (2) predominating growth of beta hemolytic streptococci on initial throat culture; and (3) an admission total leukocyte count greater than 10,000 cells per cubic millimeter. Infections were caused by Types 19 and 23 streptococci and 14 per cent of their patients had a scarlatinal rash. Significant increases in antistreptolysin 0 titer occurred in 84 per cent of untreated patients. The variability of clinical manifestations of streptococcus infections has been discussed by Rantz and his co-workers in a series of papers.[18-20] In particular they pointed out the paucity of symptoms and signs in milder cases. The criteria adopted by these workers in epidemiologic studies on a military post were: presence of large numbers of Group A streptococci in the nose or throat, and exudative tonsillitis or marked redness and edema of the soft tissues of the throat, especially when definite tender adenitis of the anterior cervical nodes was discovered. Immunologic evidence of infection was obtained in 87.5 per cent of patients classed as streptococcal infections by these criteria. This restrictive classification excluded many subclinical or mild infections, as was demonstrated during a food-borne epidemic.[20] Similar observations concerning the variability of clinical manifestations and difficulties of accurate diagnosis of sporadic streptococcus infections[14] and those encountered during a food-borne epidemic[17] have been made by the Commission on Acute Respiratory Diseases.

It is probable that no set of criteria can be derived which will accurately define every case of streptococcal infection and differentiate them from cases of nonstreptococcal etiology during seasons of high prevalence of respiratory infections in military camps. Nor is it necessary that exhaustive clinical and laboratory studies be undertaken in this attempt. As indicated by the present study, a sufficient proportion of cases can be recognized with relative ease to indicate that streptococcal infections are prevalent. Data are available which indicate that many, or even most, individuals who acquire streptococcal infections during epidemics in military camps do not voluntarily seek treatment for these infections even when they have symptoms.[3, 22] A proportion, which may be as high as 20 per cent in some epidemics, will have few if any symptoms or clinical findings and can only be detected by culture and other laboratory evidences.[17, 20, 23] Admission rates or any other rates calculated from men observed at medical installations provide only a qualitative index as to the presence of an epidemic and by no means reflect the total number of cases occurring. Since men who do not apply for treatment are probably more likely to have complications and sequelae of streptococcal infection than are those who receive adequate treatment, the occurrence of any number of cases in a military population would seem to call for institution of a prophylactic program, particularly if the risk of the program is less than is the risk of serious complications following streptococcal infection.

Where a reasonably accurate estimate of admission rates for streptococcal infections is desired, the accuracy of diagnosis in the individual cases can be greatly increased if cultures are available and the results are combined with clinical and laboratory results. Scarlet fever probably needs no further identification. Cases of exudative tonsillitis or pharyngitis with a leukocytosis and a positive culture can be considered unequivocal cases of streptococcal infection. Lacking these findings, patients with dysphagia, very tender anterior cervical nodes, or both, if also having a leukocytosis and a positive culture, can also be considered streptococcal infections. Fifty per cent or more of patients lacking all of the previously mentioned more typical features who have positive throat cultures for Group A streptococci during an epidemic can also probably be considered streptococcal infections. If the total leukocyte count is made, the percentage can be estimated more accurately. If the culture is strongly positive, or if both nose and throat cultures are positive, the accuracy of diagnosis will be further increased. These opinions are confirmed by the results obtained when 500,000 units of oral penicillin were given daily to the fourth regiment. These results showed clearly that many cases of streptococcal infection were being erroneously classified as being of non-streptococcal etiology by the criteria adopted in this study.[7] The throat culture was probably the most important single item in the diagnosis in a majority of cases and should have been afforded more weight than it was given. A recent report by Breese and Disney[24] has also emphasized the necessity of throat cultures for differentiation of streptococcal and non-streptococcal respiratory infections. These experienced observers working with children and utilizing all data available, including knowledge of streptococcal infections in other members of the family, were able to predict the results of the throat culture in only 75 per cent of patients thought to have streptococcal infections and 77 per cent of patients thought not to have them. Since positive cultures were rarely obtained from healthy children and it was probable that nearly all of the positive cultures represented infection, the culture was the most important single element in a correct diagnosis.

Maxted[25] has reported a method for differentiation of Group A streptococci from other beta hemolytic streptococci by use of bacitracin-impregnated disks on the surface of blood agar plates. His results have been confirmed in this laboratory and the method appears to be nearly as accurate as serologic identification in routine laboratory screening of freshly isolated strains of streptococci.[26] This advance further simplifies the cultural methods for identification of Group A streptococci and brings identification of these organisms within the reach of any laboratory.

SUMMARY AND CONCLUSIONS

An epidemic of streptococcal respiratory infections occurred at a Naval Training Center during the winter of 1952 to 1953. Intercurrent epidemics of influenza A and rubella also occurred. The recruit population was most severely involved by the streptococcal and rubella epidemics. Other respira-

tory infections were also prevalent in the recruit population throughout the winter. Prior to February, a Type 19 streptococcus was the most prevalent type. In the spring, a Type 3 streptococcus also became prevalent.

Scarlet fever was a prominent manifestation of Type 19 streptococcal infection but rarely occurred with other types. Exudative tonsillitis or pharyngitis was another prominent manifestation of streptococcal infections and was not related to any particular type of streptococcus. It was closely related to the presence of tonsils, being four times as frequent in patients with tonsils as in those without. Despite the frequency of occurrence of scarlet fever and exudative tonsillitis or pharyngitis, these two more easily recognizable manifestations of streptococcal infection occurred in less than one-half of the cases classed as such.

In streptococcal infections having neither a scarlatinal rash nor exudative lesions in the throat, the results of the throat culture for Group A streptococci was the single most important distinguishing feature. When the results of the culture were combined with one or more of the findings of dysphagia, very tender anterior cervical nodes, or a total leukocyte count of 12,000 cells per cubic millimeter or higher, the diagnosis of streptococcal infection was made within reasonable limits of accuracy. Even in the absence of these laboratory and clinical evidences of infection, it appeared that one-half of the cases of respiratory infection with a positive culture were of streptococcal etiology. Percentage differences existed between the frequency of such findings as high fever, malaise, myalgia, sore throat, and tender cervical nodes in respiratory infections of streptococcal and nonstreptococcal etiology. All of these occurred with sufficient frequency in nonstreptococcal respiratory infections, however, as to make them of little differential value in the individual case. This was also true of a leukocytosis of any degree less than 16,000 cells per cubic millimeter. A strongly positive throat culture was more often associated with evidences of infection than was a weakly positive culture but even a weakly positive culture was of considerable importance.

Oral penicillin prophylaxis with a dose of 250,000 units twice daily virtually eliminated admission of patients with positive cultures, scarlet fever, and exudative tonsillitis or pharyngitis. Prophylaxis with a dose of 50,000 units once daily reduced the number of admissions with evidences of streptococcal infection and seemed to reduce the degree of positivity in those with positive cultures. Neither dose had any effect on the frequency of selected clinical and laboratory findings in patients with acute respiratory infections and negative cultures or on the incidence of rubella.

REFERENCES

1. Rammelkamp, C. H., Jr., Wannamaker, L. W., and Denny, F. W.: Epidemiology and Prevention of Rheumatic Fever, Bull. New York Acad. Med. **28**: 321-334, 1952.
2. Gezon, H. M., Cook, J. S., Magoffin, R. L., and Miller, C. H.: The Use of Penicillin and Sulfadiazine as Prophylactic Agents Against Streptococcal and Non-specific Respiratory Infections Among Recruits at a Naval Training Center, Am. J. Hyg. **57**: 71-100, 1953.

3. Personnel of Naval Medical Research Unit No. 4: The Use of Triethylene Glycol Vapor for the Control of Acute Respiratory Diseases in Navy Recruits. II. Effect on Respiratory Diseases, Am. J. Hyg. **55**: 215-229, 1952.
4. Personnel of Naval Medical Research Unit No. 4: The Prophylaxis of Acute Respiratory Infections With Oral Penicillin or Chlortetracycline, Antibiotics Annual, New York, 1953-54, Medical Encyclopedia, Inc., pp. 123-136.
5. Wannamaker, L. W., Denny, F. W., Perry, W. D., Rammelkamp, C. H., Jr., Eckhardt, G. C., Houser, H. B., and Hahn, E. O.: The Effect of Penicillin Prophylaxis on Streptococcal Disease Rates and the Carrier State, New England J. Med. **249**: 1-7, 1953.
6. Seal, J. R., and Personnel of Naval Medical Research Unit No. 4: Further Studies on Penicillin Prophylaxis of Streptococcal Infections. Research Project Report NM 005 051.15.03. (To be published.)
7. Seal, J. R., Mogabgab, W. J., Friou, G. J., and Banta, J. E.: Penicillin Prophylaxis of Epidemic Streptococcal Infections. II. Comparison of Effects of Small and Large Doses of Oral Penicillin on Epidemic Streptococcal Infections and on Carriers of Group A Streptococci, J. LAB. & CLIN. MED. (In press.)
8. Swift, H. F., Wilson, A. T., and Lancefield, R. C.: Typing Group A Streptococci by M Precipitin Reactions in Capillary Pipettes, J. Exper. Med. **78**: 127-133, 1943.
9. Frank, P. F., Wilcox, C., and Finland, M.: In Vitro Sensitivity of Coliform Bacilli to Seven Antibiotics (Penicillin, Streptomycin, Polymyxin, Aerosporin, Aureomycin, and Chloromycetin), J. LAB. AND CLIN. MED. **35**: 188-193, 1950.
10. Cook, J. S., Banta, J. E., Myers, D. M., Kerby, C. C., and Forsythe, J. J.: The Epidemiological Examination of an Outbreak of Scarlet Fever at the U. S. Naval Training Center, Bainbridge, Maryland. Research Project Report NM 005 071.02, U. S. Naval Medical School, Bethesda, Md., August 22, 1952.
11. Haight, T. H.: Erythromycin Therapy of Respiratory Infections. I. Controlled Studies on the Comparative Efficacy of Erythromycin and Penicillin in Scarlet Fever, J. LAB. AND CLIN. MED. **43**: 15-30, 1954.
12. Sartwell, P. E.: Common Respiratory Disease in Recruits, Am. J. Hyg. **53**: 224-235, 1951.
13. Commission on Acute Respiratory Diseases. Acute Respiratory Disease Among New Recruits, Am. J. Pub. Health **36**: 439-450, 1946.
14. Commission on Acute Respiratory Diseases. Role of Beta Hemolytic Streptococci in Common Respiratory Disease, Am. J. Pub. Health **35**: 675-682, 1945.
15. Streptococcal Infections, Maclyn McCarty, Editor. New York Academy of Medicine, Section on Microbiology, Symposium Number Seven, Columbia University Press, New York, 1954.
16. Commission on Acute Respiratory Diseases, Exudative Tonsillitis and Pharyngitis of Unknown Cause, J. A. M. A. **133**: 588-593, 1947.
17. Commission on Acute Respiratory Diseases. A Study of a Food-Borne Epidemic of Tonsillitis and Pharyngitis Due to Beta-Hemolytic Streptococcus, Type 5, Bull. Johns Hopkins Hosp. **77**: 143-210, 1945.
18. Rantz, L. A.: The Natural History of Hemolytic Streptococcus Sore Throat, California Med. **65**: 1-20, 1946.
19. Rantz, L. A., Boisvert, P. J., and Spink, W. W.: Hemolytic Streptococcal and Nonstreptococcal Respiratory Disease. A Comparative Clinical Study, Arch. Int. Med. **78**: 369-386, 1946.
20. Rantz, L. A., Spink, W. W., and Boisvert, P. J.: Hemolytic Streptococcus Sore Throat. Detailed Study of the Simultaneous Infection of a Large Number of Men by a Single Type, Arch. Int. Med. **76**: 278-283, 1945.
21. Loge, J. P., and Kilbourne, E. D.: Penicillin Treatment of Streptococcal Pharyngitis, Ann. Int. Med. **29**: 698-714, 1948.
22. Lemon, H. M., and Hamburger, M., Jr.: Missed Cases and Contact Carriers Among Nasal Carriers of Beta-Hemolytic Streptococci, J. Immunol. **54**: 189-196, 1946.
23. Kuttner, A. G., and Krumweide, E.: Observations on the Effect of Streptococcal Upper Respiratory Infections on Rheumatic Children: A Three Year Study, J. Clin. Invest. **20**: 273-287, 1941.
24. Breese, B. B., and Disney, F. A.: The Accuracy of Diagnosis of Beta Streptococcal Infections on Clinical Grounds, J. Pediat. **44**: 670-673, 1954.
25. Maxted, W. R.: The Use of Bacitracin for Identifying Group A Hemolytic Streptococci, J. Clin. Path. **6**: 224-226, 1953.
26. Levinson, M. L., and Frank, P. F.: Differentiation of Group A From Other Beta-Hemolytic Streptococci With Bacitracin. (To be published.)

LABORATORY METHODS

AN IMPROVED METHOD OF PHOTOMICROGRAPHY BY USE OF THE ELECTRONIC FLASH

MICHAEL T. F. CARPENDALE, M.R.C.S., L.R.C.P.,* AND
EDWARD J. BALDES, PH.D.
ROCHESTER, MINN.

THE use of the electronic flash tube as a source of illumination was a fundamental advance in the science of photomicrography. It was reported by Richards in 1947,[1] although at that time it was still an experimental procedure requiring elaborate apparatus.

This paper describes an improved apparatus and method which has been designed and used by us. In practice it has proved simple to operate, adaptable to a variety of procedures, and capable of the most exacting work in all fields of photomicrography.

APPARATUS AND METHODS

Apparatus.—The apparatus described was designed originally in an atttempt to improve an existing method of taking capillary photomicrographs.[2] Subsequently, it was adapted for use with transmitted light and for phase contrast work. The apparatus consists essentially of four parts—flash unit, microscope, camera, and exposure meter.

Flash Unit.—This unit consists of a Kemlite flash tube, type Dx, and a General Electric incandescent lamp, #1493, connected by about 6 feet of flex to the power unit and switch.

The incandescent lamp is mounted at right angles to the axis of a hollow metal cylinder (1½ inches in diameter and 6 inches long) with the filament of the lamp (2 mm.) centered in the axis of the cylinder. The flash tube is mounted axially in the cylinder so that it will flash through the glass bulb of the incandescent focusing lamp, a ground glass screen, and an iris diaphragm (Fig. 1).

The power unit consists of two portions—one for the flash tube and the other for the incandescent lamp, with a rheostat to adjust the intensity. The circuit diagram of the whole unit is shown in Fig. 2.

Microscope.—Reflected light: The adjustable stand and arm from an American Optical stereoscopic microscope was used in conjunction with a Zeiss microscope body. A Leitz Ultropak with objectives 3.8×, 6.5×, and 11× facilitated the examination of objects by reflected light. The flash unit could be screwed onto the Ultropak and the amount and quality of incident light on the object adjusted by means of a diaphragm and filter (Fig. 3).

By means of a connecting tube, the camera unit could be inserted into the microscope eyepiece.

Transmitted light: A Zeiss phase contrast substage condenser and objectives (90×, 40×, and 20×) were attached to the body of a Zeiss microscope. A Leitz periplan eyepiece (10×) was

From the Section of Biophysics and Biophysical Research, Mayo Clinic and Mayo Foundation, Rochester, Minn. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

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*Fellow in Physical Medicine, Mayo Foundation.

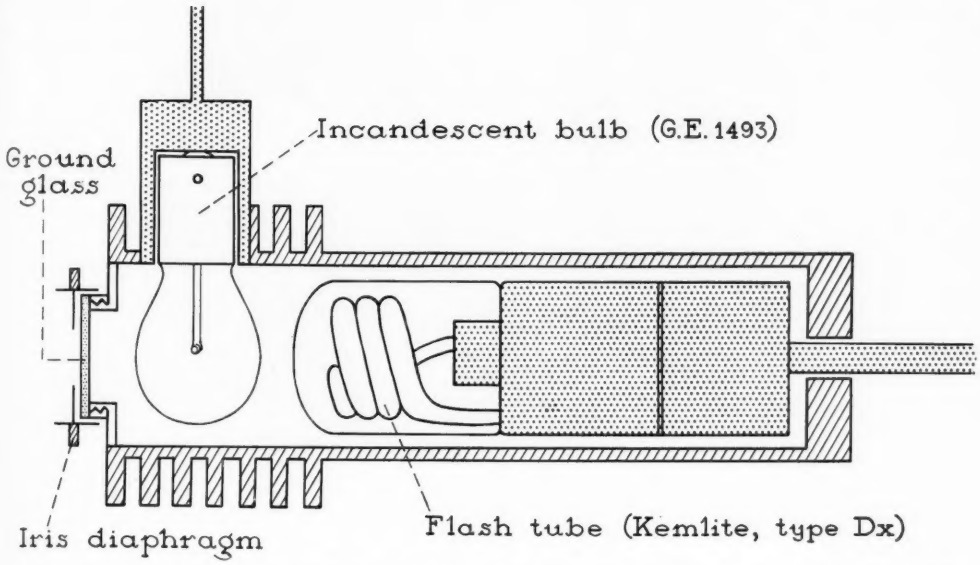

Fig. 1.—Flash unit. Note that the flash tube is mounted axially and shines through the envelope of the incandescent bulb.

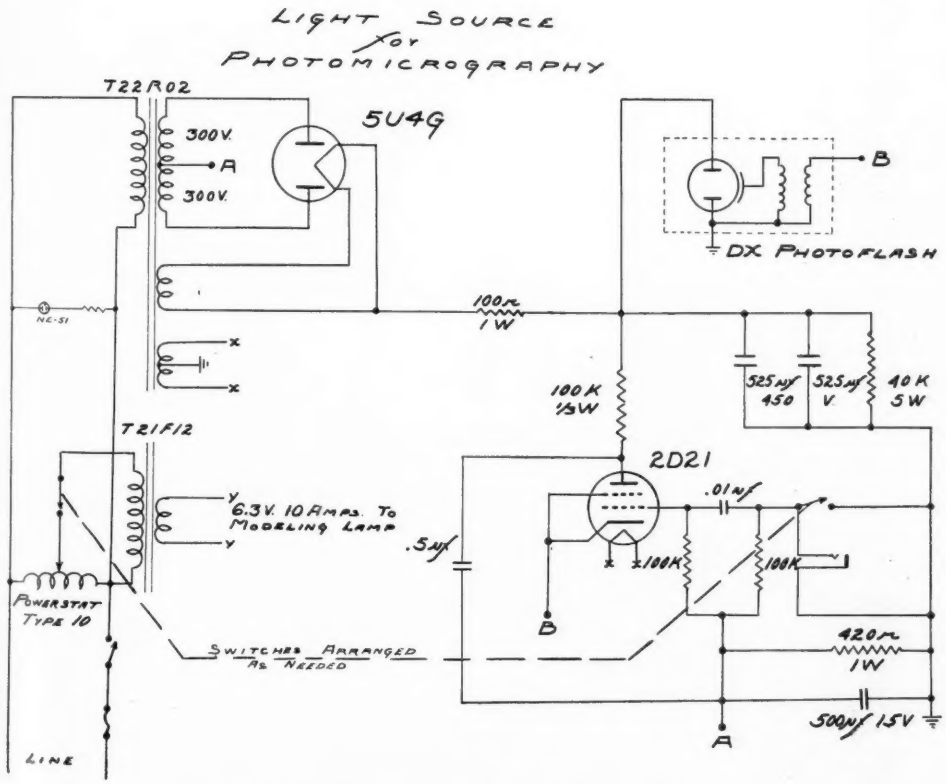

Fig. 2.—Circuit diagram for flash and power unit.

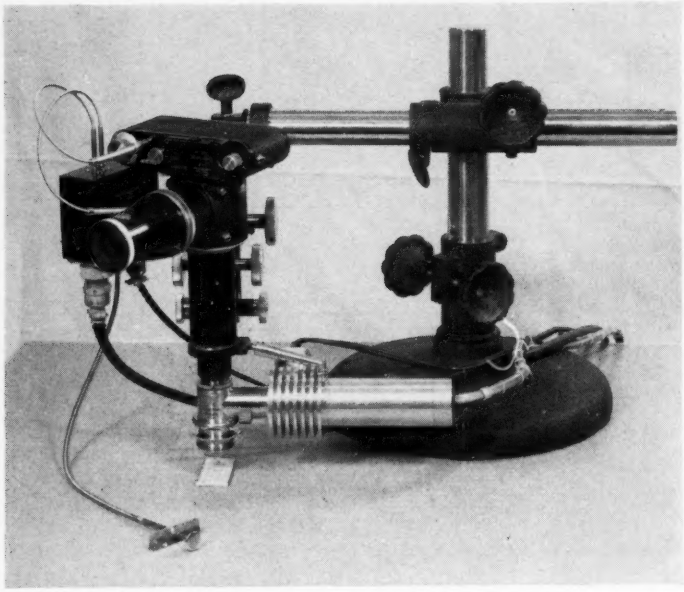

Fig. 3.—Apparatus for photomicrography by reflected light, showing camera unit, Leitz Ultropak, flash unit, and adjustable stand.

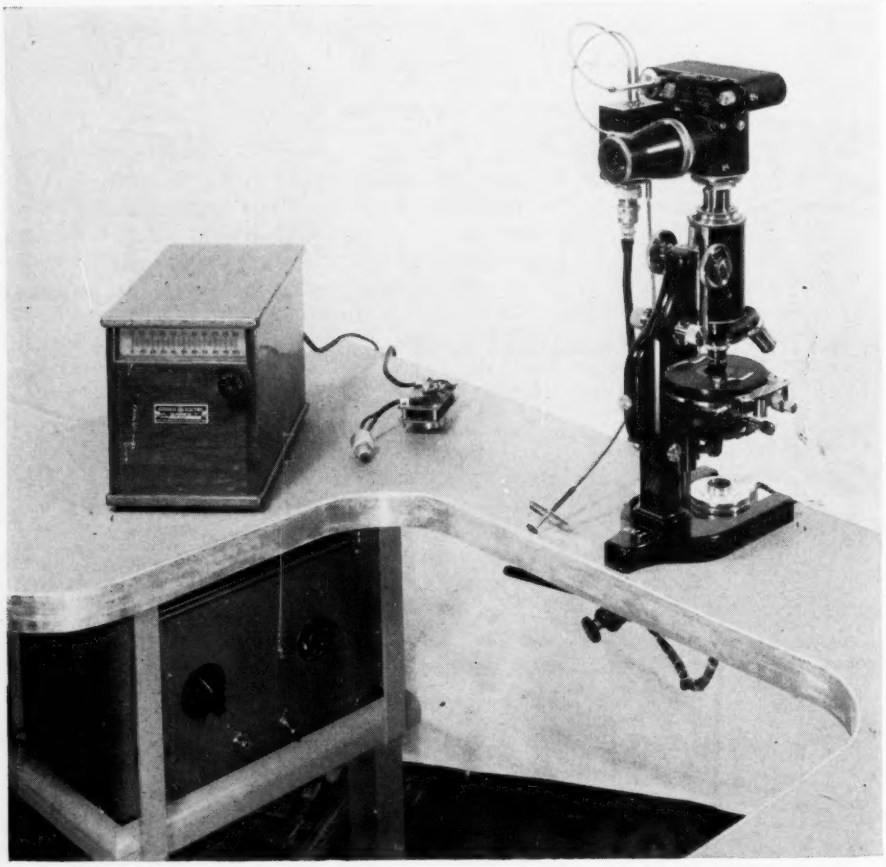

Fig. 4.—Apparatus for photomicrography by transmitted light, showing exposure meter, power unit, camera, microscope, and flash unit.

used for preliminary focusing and then attached to the camera unit. The microscope was mounted vertically on a table and centered over an aperture through which the flash unit projected a beam onto the substage condenser. The distance between the flash unit and the microscope could be altered by means of a sliding screw under the table (Fig. 4).

Camera.—The camera unit consisted of camera, reflex viewing attachment, and switch. The camera was a Leica IIIa. To this the Leitz Aristophot reflex viewfinder was fixed. An adaptor enables this to be attached direct to the eyepiece of the microscope or to a tube which can replace the eyepiece. The viewfinder, when attached direct to the eyepiece, allows visualization of the central one-third of the image which will appear on the film. The viewfinder image is magnified 15× and facilitates very accurate focusing.

The switch was arranged so that actuating the cable release performed the following actions: (1) broke the circuit to the focusing lamp; (2) raised the reflex mirror; (3) opened the shutter and (4) closed the circuit to produce a flash.

Satisfactory work has been done with a separate switch for the flash unit and an ordinary cable release for the camera, but where speed is important a mechanism such as described is an advantage.

Exposure Meter.—A General Electric photovoltaic cell coupled to a General Electric galvanometer (Cat. 32 C. 231 G 9) was used to determine the light intensity necessary for correct exposure. First, the incandescent light operating on 6.0 volts was switched on and the rheostat was turned up to give maximal intensity. Second, the photovoltaic cell was placed in the position of the eyepiece of the microscope. Finally, by means of neutral filters inserted between the illuminant and the substage condenser, the light intensity on the object was adjusted to give a known galvanometer deflection. The requisite galvanometer reading for each type of film had been determined by making a strip of test exposures with each type of film to be used. It is clear that the intensity of light from the incandescent lamp on the object is reduced by the insertion of neutral filters between them. Likewise, the intensity of the flash also passing through the same filters would suffer a comparable reduction in intensity. If now a series of test exposures reveals that, for instance, Kodachrome for adequate exposure requires a galvanometer reading of 40 and a given object under illumination produces a galvanometer reading of 60, then when neutral filters are introduced between the light and the object to reduce the reading to 40, a *normal* exposure from the use of the flash may be anticipated.

RESULTS

Representative types of photomicrographs obtainable are illustrated in Figs. 5 to 8.

COMMENT

Illuminants in common use for photomicrography suffer from three major defects: 1. low intensity requiring long exposures and consequently a vibration-free system and static object; 2. small and uneven light source corrected only by an auxiliary condensing system; 3. relatively high heat production compensated for by the use of heat filters.

The electronic flash, because of its extremely high intensity (about 500,000 watts) and brief duration (1/5,000 second), eliminates these defects. Photomicrographs of motile organisms can be taken under high powers without a specially vibration-free system. When flashed through a ground glass filter it forms a large, even source of illumination. In addition it emits little heat (this tube could be flashed in contact with one's skin with no adverse effect; hence, no auxiliary lens system or heat filters are required.

Moreover, the cost of a flash unit compares favorably with that of other comparably powerful illuminants, for example, the carbon arc.

Fig. 5.

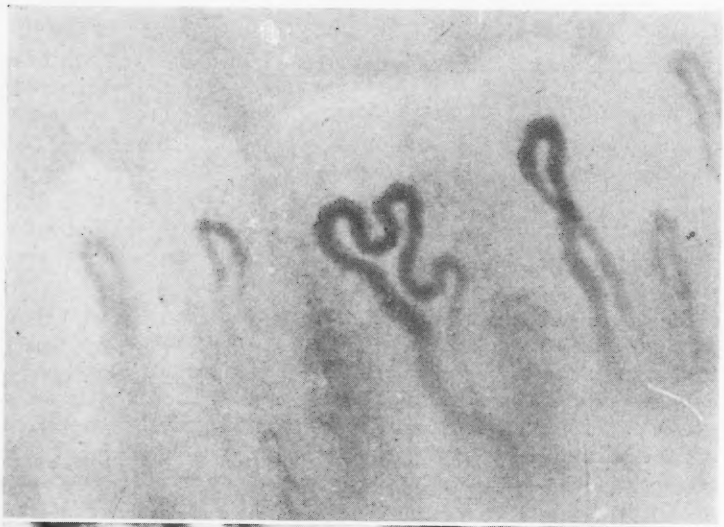

Fig. 6.

Fig. 5.—Capillaries of nailfold. Photographed by reflected light via Ultropak. Objective 11×. Total magnification 90×.

Fig. 6.—Renal tubules. Photographed by transmitted light on Kodachrome. Objective 90×. Ocular 10×.

Fig. 7.

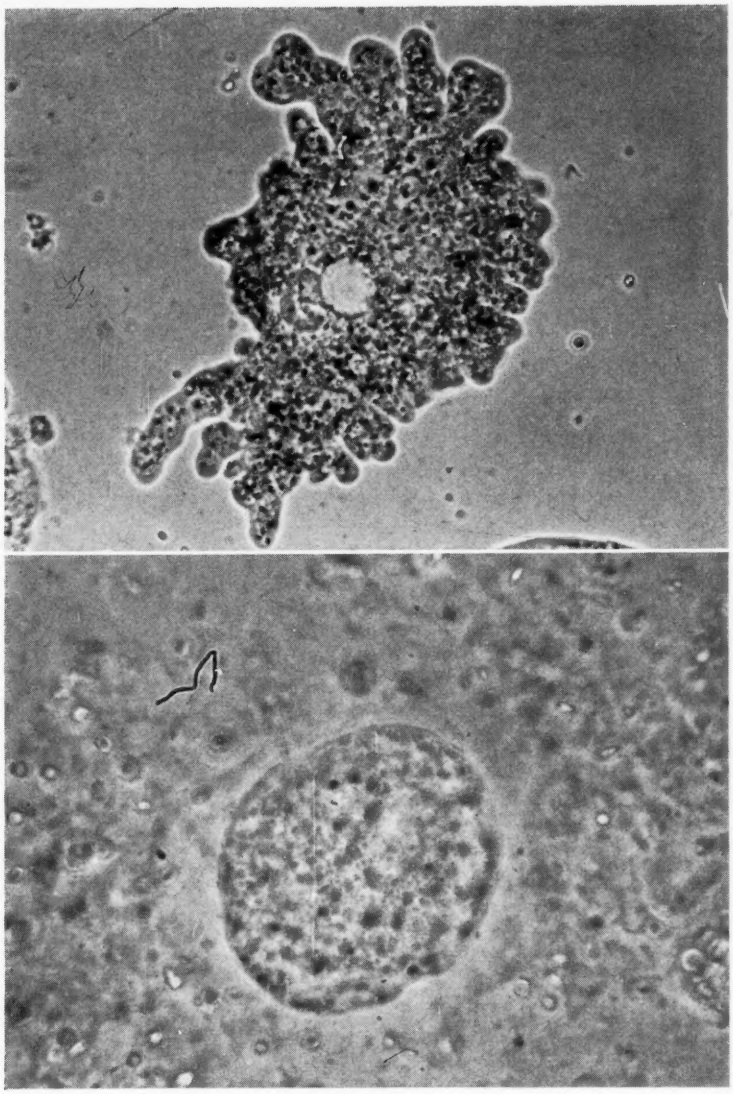

Fig. 8.

Fig. 7.—Amoeba proteus (motile). Photographed by phase contrast on Kodachrome. Objective 20×. Ocular 10×.

Fig. 8.—Nucleus of living Amoeba proteus. Photographed by phase contrast on Kodachrome. Objective 90×. Ocular 10×.

SUMMARY

An apparatus and method, using an electronic flash for photomicrography, are described.

The advantages of this illuminant are 1. high intensity, 2. brief duration, 3. even illumination, and 4. absence of heat.

These advantages simplify the technique and increase the scope of photomicrography, first, by obviating the necessity for a vibration-free system, auxiliary condensing lenses, and heat filters; and second, by enabling one to take at high powers of phase contrast high-speed color photographs of living organisms.

We wish to thank Martin Jacobs, who designed the circuit and was partly responsible for the idea of using the electronic flash, and Merlin Neher, whose technical skill and co-operation made possible the lamp housing and switch.*

REFERENCES

1. Richards, O. W.: Phase Photomicrography, J. Biol. Photographic A. **16**: 29-38, 1947.
2. Gilje, Oscar, O'Leary, P. A., and Baldes, E. J.: Capillary Microscopic Examination in Skin Diseases, Arch. Dermat. & Syph. **68**: 136-147, 1953.
3. Lonert, A. C.: Electronic Flash Illumination for Photomicrography, Turtox News **32**: 8-12, 1954.

*Since this article was written, another on the same subject has been brought to our notice. This describes some simple and relatively inexpensive apparatus, which, however, necessitates a rather complicated procedure.[3]

PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

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ABSTRACTS

1. STUDIES INVOLVING COPPER, IRON, AND ZINC METABOLISM IN SURGICAL PATIENTS

WILLIAM E. ABBOTT, M.D., STANLEY LEVEY, PH.D., HARVEY KRIEGER, M.D., AND JOHN H. DAVIS, M.D., CLEVELAND, OHIO

(INTRODUCED BY WILLIAM B. SEYMOUR, M.D.)

During a series of studies involving metabolic alterations in surgical patients it was decided to ascertain, by balance techniques, the metabolism of copper, iron, and zinc. To do this, four patients (three with pyloric obstruction requiring gastric suction and subsequent operative intervention and one with intestinal obstruction who responded to long tube intestinal intubation and did not need surgery) were maintained exclusively on an intravenous alimentation regimen whose copper, iron, and zinc content was determined by emission spectrography. The amounts of these substances in the excreta (urine, gastric drainage, and stool) were similarly determined during the study period. In this manner, it was possible to obtain metabolic balance studies.

Most of the patients were in positive iron balances for the entire study. This probably resulted because the intravenous regimen employed contained 2.1 to 7.7 mg. of iron per day depending on the solutions infused (dextrose, fructose, amino acids, or a fat emulsion), and thus the normal mechanism for the absorption of dietary iron was by-passed. The positive balance existed in the preoperative period of the three patients with pyloric obstruction and was not significantly altered by the surgical trauma of a subtotal gastrectomy.

The most striking changes noted in these studies were the large amounts of copper the patients lost by way of the drainage from the gastrointestinal tract. Negative balances of from 22 to 87 mg. copper per day were found, due primarily to these drainages. Production of a copper deficiency by strict dietary means is difficult since there is usually sufficient copper available in even the poorest natural diet to meet the individuals' requirements. A deficiency can result, however, from moderate to large external losses of gastrointestinal fluid.

The four patients also showed large negative balances of zinc (as great as 35 mg. per day). But the latter losses were less dependent on the amount of gastric drainage than that found for copper, because greater amounts of zinc are lost in the urine (one-tenth to one-half of the total excretion).

2. HYPERTROPHY OF SALIVARY GLANDS DURING TREATMENT OF MYXEDEMA WITH TRIIODOTHYRONINE

EDWIN C. ALBRIGHT, M.D., FRANK C. LARSON (BY INVITATION), AND WILLIAM P. DEISS (BY INVITATION), MADISON, WIS.

Recent evidence suggests that the salivary glands may participate in thyroid hormone metabolism, possibly through deiodination of thyroxine. The demonstration by Fawcett and Kirkwood of an enzyme tyrosine iodinase responsible for iodination of tyrosine to monoiodotyrosine or deiodination of the latter compound in rat submaxillary glands is pertinent in this connection. Further possible relationship is implied by the observation that in rats thyroidectomized or treated with thiouracil atrophy of the submaxillary glands occurs, which can be prevented by the administration of thyroxine. That a similar situation may exist in man is suggested by the following observations.

The patient, a white woman aged 38 years, six months following thyroiditis and subsequent thyroidectomy was judged to be myxedematous on the basis of symptoms, signs, and laboratory studies. Therapy with 3:5:3′-L-triiodothyronine was begun in dosage of 50 μg orally daily. After two doses she complained of tenderness in the area of the parotid glands. Examination disclosed both the parotid and submaxillary glands to be moderately enlarged and very tender to palpation. Upon massage of the parotids viscid, mucinous, colorless secretion was seen to be ejected from the ducts. The glandular hypertrophy persisted for about four days with gradual decrease in size and tenderness, although therapy was not discontinued. No fever or constitutional symptoms were noted throughout.

Saliva samples collected following administration of radiothyroxine intravenously were subjected to chromatographic analysis. Most of the radioactivity appearing in the saliva was in the form of iodide, as might be anticipated from previous studies on the fate of radiothyroxine administered to athyreotic subjects in whom radioiodide was recovered from the urine. Neither radiothyroxine nor radiotriiodothyronine were identified in the saliva. A small fraction of radioactivity remained unidentified.

The striking changes in the salivary glands noted clinically in this athyreotic patient coincident with the administration of triiodothyronine suggest an interrelationship between the salivary glands and the thyroid. If such relationship exists, its nature is still obscure, although the question of deiodination of thyroxine to form other compounds is not excluded.

3. COMPARISON OF THE PERSISTENCE IN THE BLOOD OF GAMMA GLOBULINS LABELED WITH S_{35} AND I_{131} IN THE SAME SUBJECTS

S. H. ARMSTRONG, JR., M.D., JOHN KUKRAL, M.S., M.D. (BY INVITATION), JEROME HERSHMAN, M.S. (BY INVITATION), KATHERINE MCLEOD, M.S. (BY INVITATION), AND JANET WOLTER, M.D. (BY INVITATION), CHICAGO, ILL.

We have recently reported apparent turnover times for albumins autosynthesized following oral S_{35} ingestion which are very considerably longer than the times found in the same subjects following intravenous injection of I_{131} labeled albumins.

This report extends these findings to the gamma globulins.

In two subjects, following ingestion of S_{35} labeled yeast, we have determined the apparent turnover times of the following four plasma protein fractions: albumins, a mixture of alpha$_1$ alpha$_2$ globulins, and gamma globulins (separated by paper electrophoresis) and fibrinogen.

Despite consistently differing blood levels of these fractions both subjects showed closely comparable apparent turnover times. For albumins these were 42 and 47 days; for globulins of intermediate mobility, 25 and 24 days; for gamma globulins, 33 and 37 days, respectively.

In a second experimental period the apparent turnover time of the gamma globulins following intravenous injection of an I_{131} labeled preparation was determined in the same subjects. The values were 19 days and 23 days, respectively, or approximately 60 per cent of the comparable S_{35} values.

The implications of these findings are discussed from standpoints of methodology and of metabolic interpretation of variations of "normal" of apparent turnover times in disease states.

Specifically, with the suspicion of reutilization of isotope from labeled body proteins following oral ingestion unsettled, and with the possibility of altered metabolic pathway for proteins subtly changed by in vitro iodinization still existent, is it as yet possible to carry off clinically significant studies on the dynamics of plasma proteins?

The type of evidence needed to settle this problem will be discussed.

4. A STUDY OF CARBOHYDRATE OXIDATION IN DIABETIC SUBJECTS BY MEANS OF C^{14}-LABELED GLUCOSE

Nome Baker, Ph.D. (By Invitation), W. W. Shreeve, M.D. (By Invitation), Max Miller, M.D., R. A. Shipley, M.D., and J. W. Craig, M.D. (By Invitation), Cleveland, Ohio

Four normal subjects and eight diabetics, temporarily deprived of insulin, were injected with 100 μc of uniformly labeled C^{14} glucose. Specific activities of blood glucose and of expired CO_2 were determined serially during the ensuing six hours. After the initial phase of diffusion the glucose curve declined in simple exponential fashion in all cases. The activity of CO_2 rose to a peak within two to three hours, and then declined slowly.

From theoretical considerations it was concluded that the glucose pool could be regarded as the sole source from which a second sizable pool consisting of CO_2—HCO_3 could derive its C^{14}. The rise and fall of CO_2 activity could be explained by the enrichment of the latter pool with C^{14} combined with a concurrent loss of CO_2 in the breath. With such a model it is possible to construct the hypothetical curve of specific activity for CO_2, assuming that oxidation of glucose is its only source. The height of the *observed* CO_2 curve may then be compared with that of the *theoretical* curve. The ratio of the two is a measure of the proportion of CO_2 contributed by the oxidation of glucose as compared to the remaining major fuels such as fat and protein.

Normal subjects gave values ranging from 17 to 29 per cent of CO_2 derived from glucose. The mean was 22 per cent. In five "stable" diabetic subjects, upon withdrawal of insulin the mean was 20 per cent with a range of 16 to 31 per cent. Three "severe" diabetics who promptly developed ketoacidosis upon withdrawal of insulin averaged 10 per cent (range 9 to 12 per cent). Calculations of milligrams of glucose per kilogram of body weight oxidized per hour averaged 38 for the normal group, and 49 and 18 for the two respective diabetic groups.

It is concluded that no defect in the *rate* of glucose oxidation was demonstrable in diabetic subjects who were not progressing into ketosis, while a distinct decrease was apparent in three patients who, during the course of the study, showed such a metabolic shift.

5. DETERMINATION OF NORMAL BLOOD VOLUMES OF 100 AMBULATORY HOSPITAL PATIENTS

ROBERT J. BAKER, M.D. (BY INVITATION), AND DONALD D. KOZOLL, M.S., M.D., CHICAGO, ILL.

The value of rapid, readily available blood volume determinations in massive gastrointestinal hemorrhage, in debilitated states, in preoperative patients, in massive burns, and in other emergent conditions, has long been appreciated. However, it should again be noted that a blood volume determination is of value only as it relates to the estimated normal volume for a given individual at a specific time.

This study was undertaken as the first part of a project to evaluate and standardize, for practical clinical usage, the procedure of blood volume determination, utilizing radioactive iodinated human serum albumin (RIHSA) administered intravenously. All counting was done on plasma samples in a well-type scintillation counter.

Normal blood volume values have been based on the patient's weight, his expected normal weight, his normal weight with obesity correction (Randall's factors), his height, his surface area, and his age and sex correlated with any or several of the preceding. Because an initial application of several of these gave such widely divergent results, and since the interpretation of results is completely dependent on variation from normal, it was decided to determine blood volume in 100 ambulatory surgical patients, on full diet and normal oral fluid intake; all patients were admitted to the Cook County Hospital for relatively minor surgical conditions.

It was immediately apparent that a simple weight-blood volume ratio was highly impractical, as all patients below 54 kg. (17 patients) had volumes higher than the expected normal (using 80 ml. per kilogram), and all patients over 54 kg., with two exceptions, volumes lower than the expected normal. The "normal" weight evaluation also created incongruous results, and, hence, the surface area (Boothby-Sandiford nomogram) was calculated; a linear relation between surface area, utilizing height and weight, and blood volume was found to be statistically valid in all instances.

The implications of the results will be discussed, as well as the practical application in fifteen illustrative cases. Any fluid, plasma, or whole blood replacement therapy governed by the blood volume determination is based on a valid calculation as to normal volume, without which the technique becomes simply comparative; an immediate guide to therapy is lacking.

6. URINARY UROPORPHYRIN, PORPHOBILINOGEN AND COPROPORPHYRIN EXCRETION IN LEAD-EXPOSED WORKERS

F. A. BASHOUR, M.D., MINNEAPOLIS, MINN.

(INTRODUCED BY C. J. WATSON, M.D.)

Urinary coproporphyrin and uroporphyrin excretion, erythrocyte protoporphyrin, blood lead concentration, basophilic stippling, basophilic aggregate, hematocrit, and hemoglobin were studied in a group of 44 industrial

workers exposed to lead. With the exception of 5 individuals with clinical complaints suggestive of lead poisoning, all were in good clinical condition at the time of study.

Most striking changes were observed in the levels of urinary and erythrocyte porphyrin. Per diem coproporphyrin excretion averaged 1,704 ± 783 μg as compared to a normal average of 189 ± 79 μg. The erythrocyte protoporphyrin values averaged 467 ± 254 (range 146-942) as compared to control values 29 ± 4.5 μg per 100 ml. Erythrocyte coproporphyrin values were only slightly elevated, all values falling in the range of 0.6 to 6.4 μg per 100 ml. The daily uroporphyrin excretion averaged 96.2 μg as compared to the normal average of 13 ± 10 μg. The uroporphyrin methyl ester was crystallized from the combined extract of 24 urine collections of 6 individuals in the lead-exposed group. The crystalline uroporphyrin methyl ester melted at 259° to 262° C. indicating the presence of at least some Type III isomer, but whether as 8 or less COOH groups was not determined. It behaved as a Type B Waldenstrom porphyrin and is receiving further study.

Blood lead concentration was elevated above the normal level of 0.06 mg. per 100 ml. in 39 individuals, while 10 had hematocrits of less than 40 per cent. Basophilic stippling was elevated in only a few instances.

Significant correlation coefficients were observed for the following tests: Basophilic stippling and lead aggregates (0.79); urine coproporphyrin and uroporphyrin (0.68); blood lead and urine coproporphyrin (0.37); blood hemoglobin and urine coproporphyrin (0.33).

Recent investigation has revealed the presence of porphobilinogen in a second group of 13 workers exposed to lead. These results are in accord with the finding of Schwartz and co-workers of the increased excretion of uroporphyrin together with coproporphyrin and porphobilinogen in experimental lead poisoning. This is the first report of excessive uroporphyrin excretion in human beings having lead exposure.

7. AN UNDESCRIBED HUMAN HEMOGLOBIN ASSOCIATED WITH HEREDITARY HEMOLYTIC ANEMIA

JOHN D. BATTLE, JR., M.D., AND LENA LEWIS, PH.D., CLEVELAND, OHIO

(INTRODUCED BY A. C. ERNSTENE, M.D.)

The presence of a mild chronic nonspherocytic hemolytic anemia with conspicuous poikilocytosis, hypochromia, and "target cells" in a 33-year-old white man prompted examination of other members of his family. A sister, aged 39 years, was the only relative available for study and she was found to have a similar type of hemolytic anemia. Both siblings revealed pallor, mild jaundice, and splenomegaly. Sickling tests were negative. The fetal hemoglobin was slightly elevated by the Singer technique. These findings suggested the possibility of the presence of an inherited abnormal hemoglobin.

The hemoglobin of each sibling was analyzed by Longsworth's modification of the Tiselius electrophoretic technique. Cacodylate buffer pH 6.5, ionic strength 0.1 μ; phosphate buffer pH 7.8, ionic strength 0.15 μ; and phosphate buffer pH 9.2, ionic strength 0.1 μ were used. In cacodylate buffer two poorly resolved components with mobilities of 2.7×10^{-5} cm./volt/sec./cm.^{2} and 2.4×10^{-5} cm./volt/sec./cm.^{2} were observed. The latter mobility is that of normal adult hemoglobin. In phosphate buffer at pH 9.2 only one component was resolved. When the patient's hemoglobin was mixed with normal hemoglobin and analyzed at pH 9.2, only one component was found. At pH 7.8, however, there was a clear resolution of 2 components of the patient's hemoglobin. The more rapid component moved with the mobility of normal hemoglobin and

comprised 25 per cent of the total. The slower moving component amounted to 75 per cent of the total. When a mixture of the patient's hemoglobin and the hemoglobin of a patient with sickle cell anemia was analyzed at pH 7.8, three peaks were separated. These components had the mobility of normal hemoglobin, Type S, and midway between normal and Type S. Filter paper electrophoretic analysis of the patient's hemoglobin at pH 8.6 was similar to a control with normal adult hemoglobin.

These studies suggest that the abnormal hemoglobin in this brother and sister is a new and hitherto undescribed form, different from the abnormal Types S, C, D, and E.

8. STUDIES ON THE FEBRILE RESPONSE FOLLOWING INTRAVENOUS ADMINISTRATION OF FAT EMULSIONS TO HUMAN SUBJECTS

GERALD H. BECKER, CAPTAIN, MC, USA (BY INVITATION), HUGO C. MOELLER, CAPTAIN, MC, USA (BY INVITATION), AND MORTON I. GROSSMAN, PH.D., M.D., DENVER, COLO.

This study attempts to correlate the incidence and magnitude of the fever following intravenous infusion of fat emulsions with the level of fat in the blood, as measured by turbidity and free and total fatty acid concentrations. A 15 per cent olive oil emulsion (Upjohn) and a 15 per cent cottonseed oil emulsion (Harvard School of Public Health) were used. Each of 8 normal young male subjects received one 500 ml. infusion of each emulsion, one week apart. The average time required for the 500 ml. infusion was 3.5 hours. Venous blood samples were drawn every 20 minutes for the first hour and then hourly until four hours after the end of the infusion. At the same times rectal temperatures, pulse and respiration rates, blood pressures, and any side reactions were recorded. Six of the 8 infusions with the olive oil emulsion resulted in thermogenic reactions of 2° F. or more, while this occurred in 3 of 8 subjects given the cottonseed oil preparation. The maximum fever occurred during the second and third hours after the end of the infusion with the former emulsion, during the first hour after the end of the infusion with the latter. No statistically significant difference was found in the magnitude or duration of fever between the two emulsions, a result which was comparable to simultaneously performed pyrogen tests in rabbits. The peak levels of turbidity and free and total fatty acids coincided with the end of the infusion. Free fatty acid levels had not returned to control levels by the fourth hour after completion of the infusion, and this persistence of elevation was independent of whether total fatty acid had returned to control levels and also independent of the occurrence or severity of febrile responses. Following those infusions which were attended with febrile responses to either emulsion, turbidity and total fatty acid levels attained lower peak values and returned to control levels more quickly in those with fever than in those without fever.

9. THE INFLUENCE OF ACTH AND CORTISONE ON THE INCIDENCE OF INFECTIONS

THOMAS G. BENEDEK, M.D., AND MAX M. MONTGOMERY, M.D., CHICAGO, ILL.

(INTRODUCED BY LOUIS R. LIMARZI, M.D.)

It has been widely assumed that the administration of ACTH and cortisone leads to increased susceptibility to infection. The present study has been undertaken to test the validity of this belief.

The case material consisted of 114 patients with rheumatoid arthritis and 31 patients with disseminated lupus erythematosus. Twenty-five arthritis patients were treated with cortisone and/or ACTH for three to twelve months and 19 for 13 to 39 months, a total of 673 months of therapy. These 44 patients were observed during 465 months of hormone therapy and for 411 months without such medication. There were 9 and 16 infections, an incidence of one every 52 months and one every 26 months, respectively.

Of the 31 lupus erythematosus patients, 10 have received cortisone and/or ACTH for one to 5½ months and 11 for 6 to 39½ months, a total of 239 months of therapy. The patients were observed during 209 months of hormone therapy and for 275 months without such medication. There were 11 infections in each period, an incidence of one every 16 months and one every 25 months, respectively. One terminal infection occurred among the 7 untreated patients who have expired, and one among the 10 untreated patients.

Hormone therapy was not associated with infections of unusual type or severity, although symptoms were masked in a few cases. Therapeutic doses of ACTH and cortisone did not exert a pronounced effect on the incidence of infections in patients with rheumatoid arthritis and disseminated lupus erythematosus. An analysis of about 900 patient-months of hormone therapy did not demonstrate significant differences from controls.

10. THE ISOLATION AND CHARACTERIZATION OF THE NONSPECIFIC HYALURONIDASE INHIBITOR OF HUMAN BLOOD

GERALD S. BERENSON, M.D., JOYCE K. NEWMAN, MARTIN B. MATHEWS, PH.D., AND ALBERT DORFMAN, M.D., CHICAGO, ILL.

(INTRODUCED BY ROBERT H. EBERT, M.D.)

There has been widespread interest in the existence in human blood of a substance which inhibits the enzyme hyaluronidase. Numerous reports have indicated that the level of the substance is increased in a wide variety of diseases including rheumatic fever. By means of a purification procedure involving alcohol fractionation, precipitation at low ionic strength and electrophoresis on a starch slab, the activity has been purified up to 1,000 fold. Preparations so isolated appeared to be essentially monodisperse on ultracentrifugation, free solution electrophoresis and diffusion studies. The molecular weight is estimated to be about 100,000. The sedimentation constant $S_{20} = 3.7$ S and mobility in veronal buffer, pH 8.6, $\mu = 0.1 = -5.4 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volt.$^{-1}$ Analyses performed showed N = 14.4 per cent, polypeptide content 75 per cent, hexosamine 4 per cent, S < 0.1 per cent, P < 0.2 per cent, tyrosine 2.7 per cent, and tryptophane 0.9 per cent. A positive test for hexuronic acid was obtained by the carbazole method. The following amino acids were demonstrated by paper chromatography: cysteic acid, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, lysine, arginine, valine and/or methionine, and luceine and/or isoleucine.

11. EXPERIENCES WITH THE SCHILLING TEST USING CO^{60} LABELED VITAMIN B_{12} IN PERNICIOUS ANEMIA, SPRUE, AND OTHER CONDITIONS

WILLIAM R. BEST, M.D., WILFRED F. WHITE (BY INVITATION), JOHN LOUIS, M.D. (BY INVITATION), AND LOUIS R. LIMARZI, M.D., CHICAGO, ILL.

Schilling demonstrated significant urinary radioactivity in normals when 2 μg $B_{12}CO^{60}$ orally were followed in two hours by 1.0 mg. B_{12} parenterally (J. LAB. & CLIN. MED. 42: 860, 942, 1953). We have employed this test or

modifications 56 times in 21 patients. Percentage equivalent of oral dose in 24-hour urine was 9.2 to 14.4 per cent in four controls, 0.2 to 2.0 per cent in 10 PA's (pernicious anemia patients), 11.7 to 18.7 per cent (except one of 40.0 per cent) in 8 PA's receiving potent doses of intrinsic factor concentrate (IFC) mixed with $B_{12}CO^{60}$, 1.2 and 2.2 per cent in two sprue patients, and 2.5 and 3.5 per cent, respectively, when potent IFC was also given. Replicate tests with good recoveries in three PA's and one control showed excellent agreement in two and poor agreement, possibly related to oliguria, in two. Graded doses of IFC in three other PA's gave 24-hour excretions which could be fitted quite well to log-dose or exponential asymptotic curves. Activity of IFC preparations by this test agreed qualitatively with U.S.P. clinical assays.

Fractional urinary collections revealed faint radioactivity during the first two hours unrelated to the presence of IFC. There was generally a delayed increase to a single peak value at a time characteristic for the individual, usually between 8 and 12 hours, always more than 6 hours after the oral dose. Peak values were equivalent to 17.3 to 34.00 mμg $B_{12}CO^{60}$ per hour in the controls and PA's receiving potent IFC (except one PA with 51.2). In most cases excretion rates were very low by 24 hours.

Administration of B_{12} two hours before or simultaneously with $B_{12}CO^{60}$ did not qualitatively alter the results. B_{12} plus IFC is not absorbed when administered rectally.

From evidence available it is speculated that B_{12} combines with IFC by mass action, that specific portions of the gastrointestinal mucosa assimilate this combination at fixed maximal rates, that a period of six or more hours is necessary to metabolically traverse the mucosa, and that free B_{12} is ultimately released into the portal circulation where it becomes bound to protein. It appears probable that some modification of this test may prove superior for the assay of intrinsic factor preparations.

12. THE CALORIC EQUIVALENT OF WEIGHT LOSS

WILLIAM R. BEST, M.D., CHICAGO, ILL.

The caloric deficit necessary to produce a given weight loss has been previously estimated from metabolic studies on a small number of subjects. Estimates of caloric expenditure are crude at best. In the course of a large-scale Army field experiment for another purpose (J. Clin. Nutrition 2: 97, 1954) data became available to estimate this relationship in a unique way. During two separate three-week periods 81 men were subjected to rigidly controlled diets and fairly uniform conditions of exposure to winter weather and vigorous physical activity. Mean measured values for the first and second periods, respectively, were: caloric intake (CI), 3,453.4 and 2,263.5 calories per day; specific dynamic action (SDA), 7.3 and 7.0 per cent of intake; average body weight (BW), 70.8 and 68.6 kg.; and weight loss (WL), 0.027 and 0.190 kilogram per day. Assuming a uniform ratio (k) of weight loss to caloric deficit and a constant caloric expenditure per kilogram body weight in excess of SDA (CE), the following formula may be written:

$$WL = k(BW \times CE + SDA - CI).$$

By substitution of observed WL, BW, SDA, and CI for the two periods, k and CE may be derived through solution of simultaneous equations.

In this instance CE is calculated to be 47.5 calories per kilogram per day, and k to be 0.000164 kilogram per calorie deficit. This latter may be expressed

as 6.1 calories per gram. If caloric expenditure is considered constant and unrelated to body weight, a value of 6.7 calories per gram is obtained. The truth probably lies somewhere between these assumptions. Work has been cited (Phys. Rev. 33: 311, 1953) indicating that tissue lost during caloric deficit is approximately 65 per cent fat, 23 per cent cellular matter, and 12 per cent extracellular fluid. If caloric factors of 9, 4, and 0 are assigned to these components, respectively, a value of 6.8 calories per gram is arrived at; in good agreement with the present experimental value.

13. THE PLASMA CLEARANCE OF HEMOGLOBIN IN NORMAL AND NEPHRECTOMIZED RATS

W. F. Bethard, M.D., and L. Plzak (By Invitation), La Jolla, Calif.

Solutions of labeled hemoglobin, prepared by laking erythrocytes from Srpague-Dawley rats previously injected with radioiron, were administered intravenously in varying concentrations to groups of normal and nephrectomized recipient rats. Blood samples were taken at appropriate time intervals after injection to permit calculation of plasma-clearance rates, and selected organs were analyzed for radioiron at the time of sacrifice. Carcass radioiron determinations were not done, and no attempt was made at quantitative recovery. Injection volume was kept constant.

Plotted semilogarithmically as a function of time, the data yielded straight lines which could be expressed mathematically by the usual equation for first order reactions. Because the amount of hemoglobin cleared in a given time was proportional to the amount available, results were normalized to the amount theoretically cleared when 100 milligrams of hemoglobin were present.

Experiments were performed using 39, 56, and 100 mg. of hemoglobin. The average plasma clearance rates in nephrectomized animals were 29.8, 38.2, and 28.1 mg. per hour per 100 mg. hemoglobin, respectively, and controls cleared 92.9, 93.4, and 84.7 mg. per hour per 100 mg. hemoglobin. In each case plasma-clearance rates in nephrectomized animals were 30 to 40 per cent of those in normal controls. Apparently tissues other than kidney can remove noncolloidal, nonnative protein from plasma at a relatively rapid rate.

About three hours post injection all animals were sacrificed and the following tissues were analyzed for radioiron: stomach, intestines, liver, spleen, lymph node, lungs, kidneys, brain, and femur. Data on hemoglobin uptake by these tissues will be presented. Average total recovery in the organs examined was 21.3 per cent for the nephrectomized group and 26.3 per cent for the controls.

14. COMPARATIVE EFFECTIVENESS OF DAILY DOSES OF L-TRIIODOTHYRONINE OR OF L-THYROXINE IN THE CONTROL OF MYXEDEMA

Charles M. Blackburn, M.D. (By Invitation), and F. Raymond Keating, Jr., M.D., Rochester, Minn.

Until the recent discovery of triiodothyronine, no other iodinated amino acid had been found which possessed more than a fraction of the calorigenic activity of thyroxine. Triiodothyronine, however, on the basis of some bioassays, was found to be more calorigenic than thyroxine on an equimolar basis by a factor of 4 or 5.

The present study was undertaken to compare the ability of daily oral or intravenous doses of l-thyroxine and l-triiodothyronine to maintain a euthyroid state and a normal metabolic rate in athyreotic patients over an extended period.

In ten such patients the daily maintenance dose of l-triiodothyronine when taken by mouth was found to vary from 52 to 105 μg, whereas in 4 of these patients the maintenance dose of l-thyroxine by mouth varied from 175 to 400 μg. On this basis, l-triiodothyronine appears to be three to four times more potent than l-thyroxine.

The effect of oral versus intravenous administration of l-triiodothyronine was compared in five patients and that of oral versus intravenous administration of l-thyroxine was compared in three patients. L-triiodothyronine was only slightly more effective when given intravenously than when used orally, whereas l-thyroxine was twice as potent by the intravenous route as when given orally.

Two patients were maintained at a normal and stable metabolic level and in a clinically euthyroid state for one year with daily doses of l-triiodothyronine administered orally, indicating that this agent does not become physiologically less active with the passage of time, and that adequate control of myxedema can be accomplished indefinitely by continued administration of the agent. No clinical evidence was encountered which would suggest that triiodothyronine is qualitatively superior to desiccated thyroid substance in the treatment of myxedema.

15. DEMONSTRATION OF DIGLYCERIDES IN HUMAN INTESTINAL CONTENTS DURING FAT DIGESTION

D. H. Blankenhorn, M.D., and E. H. Ahrens Jr., M.D., Cincinnati, Ohio

(Introduced by Morton Hamburger, M.D.)

In order to achieve better definition of the complex relationships within the human intestinal lumen during fat digestion, it seemed desirable to sample the gut contents at various levels after feeding balanced test meals containing finely homogenized fat of known chemical composition. For purposes of localized sampling, transintestinal intubation with 2 mm. polyethylene tubing had definite advantages over previous methods and was well tolerated in 6 patients for 4 to 21 days continuously. Subjects showed no apparent disturbance in gastrointestinal function, slept well, and maintained weight. The technique permitted reproducible measurement of the length of the various portions of the intestine, and for aspiration of gut contents holes in the tube could be brought to any desired position. Although the tubing was sufficiently flexible to conform to the normal convolutions of the gut in x-ray studies, it did not collapse under constant suction (12 cm. Hg). Test meals consisted of liquid homogenates of protein, carbohydrate, and defined fats. Taken orally in 4 to 6 feedings per day, such formulas furnished the sole nutriment of 30 patients for 2 to 12 weeks. Fixed intakes of all food variables were thus assured; body weights were remarkably constant. Intubated subjects were conditioned to oral formula feeding for several days prior to aspiration of gut contents for chemical study.

Three experiments in 2 normal subjects have been completed in which the hydrolytic products of triglyceride digestion in the gut were isolated by countercurrent distribution and conclusively identified. Diglycerides were

demonstrated for the first time in human experiments. Monoglycerides, diglycerides, triglycerides, and fatty acids were found when aspirations were made at two positions in the upper jejunum, while at the ligament of Treitz no hydrolytic products were demonstrable. Control analyses established the homogeneity of the test triglyceride and showed that in vitro hydrolysis of the test fat in the presence of intestinal enzymes during storage and laboratory manipulation had not occurred.

16. HISTOPATHOLOGIC BASIS FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA IN ANEMIC PATIENTS AND FOR COMBINED TREATMENT WITH TRANSFUSIONS, RADIOPHOSPHORUS, AND INTRAVENOUS IRON (EXCEPT IN THE BURNT-OUT LEUKEMOID STAGE)

MATTHEW BLOCK, M.D., PH.D., WILLIAM BETHARD, M.D., AND LEON O. JACOBSON, M.D., DENVER, COLO.

Patients with polycythemia vera who are also anemic because of spontaneous hemorrhage (usually gastrointestinal) are encountered frequently. Thromboses, intractable or recurrent upper gastrointestinal ulcers, and pruritus often accompany the hemorrhagic phenomena. These patients present difficult diagnostic and therapeutic problems. Diagnosis may be made regardless of the degree of anemia by recognition of a probably pathognomonic appearance of sections of marrow that show extreme panhyperplasia with complete absence of iron, as stained with Prussian blue. Megakaryocytes are particularly numerous. Nonspecific red pulp hyperplasia, always without demonstrable stainable iron and usually with slight myeloid metaplasia, is found in the spleen.

Paradoxically, these manifestations of activity of the disease may be controlled by injection of 6 to 8 mc. of radiophosphorus and iron given intravenously and by transfusions. Successful control of the coincidental hemorrhagic and thrombotic complications and gastrointestinal ulcers parallels and possibly depends upon a decrease in the hypercellularity of the marrow induced by radiophosphorus since complete remissions coincide with a reduction of marrow to the lower limit of normal cellularity (one-half to two-thirds fat) as judged from the sections. Dosages that do not bring about this reduction in cellularity usually permit persistent activity that is manifested by hemorrhagic phenomena, gastrointestinal ulcers, and pruritus. Since anemia, except in the burnt-out leukemoid stage, is considered a complication of uncontrolled polycythemia, it is an indication, not a contraindication, for therapy directed toward inhibition of the marrow.

The primary utilization of iron for erythropoiesis, the absence of stainable iron in hematopoietic tissues, and the hypochromic, microcytic characteristics of the red cells are evidences of severe iron deficiency, which has been treated successfully, most rapidly, and with least gastrointestinal distress with intravenously-administered iron.

Transfusions exert a direct, immediate beneficial effect upon the decreased blood volume, but are probably not adequate therapy per se since they do not decrease the activity of the polycythemia. They do not interfere with the radiophosphorus and iron, which exert a delayed effect upon the peripheral blood via the bone marrow.

17. THE TRANSMISSION OF THE PULMONARY ARTERY PRESSURE ACROSS THE CAPILLARY BED

S. Gilbert Blount, Jr., M.D., Geffredo Gensini (By Invitation), and Oscar Balchum (By Invitation), Denver, Colo.

Pulmonary venous wedged pressures (PVWP) were obtained in six patients, including three with atrial septal defects and two with anomalous connections of pulmonary veins to the right atrium. The sixth patient with valvulvar pulmonic stenosis and a patent foramen oval was studied both prior to and following pulmonic valvulotomy.

The PVWP closely resembled the pulmonary artery pressure contours in all instances. Where comparison was possible the pressure levels and contours of the PVWP were very similar to the pulmonary artery pressure levels and contours. The resistance of the pulmonary vasculature and the magnitude of the pulmonary blood flow apparently affected the transmission of the pulmonary artery pressure to an insignificant extent within the limits of this study.

In pulmonary valvular stenosis the PVWP was greater than the pulmonary artery pressure. This is probably due to the greater degree of stenosis that exists when the cardiac catheter further constricts an already stenotic pulmonary valvular orifice. Therefore, it would appear that the PVWP is a more accurate estimation of the pressure actually existing in the pulmonary artery in the case of pulmonic stenosis.

18. CLINICAL AND PHYSIOLOGIC EVALUATION OF SEVERAL ORALLY EFFECTIVE BRONCHODILATORS

Hollis G. Boren, M.D., Demitri J. George, Irving C. Chofnas, M.D., and Carroll A. Handley, Ph.D., Houston, Tex.

(Introduced by Charles L. Spurr, M.D.)

Three N-substituted arterenol derivatives have been evaluated following their oral administration. Twenty-four male patients, averaging 58 years of age, with continuing difficulty due to pulmonary emphysema and chronic bronchitis, despite the usual regimen, were studied with special emphasis on pulmonary function. Toxic effects were limited to occasional slight decrease of blood pressure and tachycardia accompanied at times by palpitation or dizziness. No laboratory evidence of hematological, hepatic, electrocardiographic or renal damage was found. Wheezing, present in 13 cases, decreased markedly in 11. Eleven cases on maintenance drugs for several weeks showed no toxicity. Moderate to marked increase of vital capacity was frequently found. Timed vital capacity showed increases in volumes expired in 1, 2, and 3 seconds which were proportional to the increase of total vital capacity. Nitrogen elimination curves only infrequently showed a decrease of time required to reach a minimal value. Maximum breathing capacities were prohibited by the condition of many of the patients.

The effects of these drugs were best shown by a study of mechanics of ventilation. Lung surface pressure was measured by an intraesophageal catheter, flow by the Lilly flowmeter, and volume by an electrically recording spirometer. Changes were recorded on a multichannel electrocardiograph and an oscilloscope. Mechanics studies on 8 patients showed no or slight decrease of frequency of breathing, significant decrease of the extremes of pressure

fluctuation in 5 of 7 cases, volume and flow increase in half the cases, and significant decrease of resistance in 3 of 4 patients on whom resistance measurements were reliable. Compliance studies on 5 patients showed an increase in 2, decrease in 1, and no change in 2 patients. Despite compliance increase, resistance decreased. Of the three drugs studied, two seem to be effective bronchodilators and have insignificant toxic effects. They are N-[2-(1-p-methoxyphenylisopropyl)] norepinephrine hydrochloride (JB245, Lakeside) and N-[2-(3,4-methylenedioxyphenylisopropyl)] norepinephrine hydrochloride (JB-251, Lakeside). The effective dose of these drugs is 2 to 5 mg. with onset of action within 15 minutes and duration of action 2 to 4 hours. It has not been necessary to give any of these drugs sublingually to produce significant effect.

19. THE PATHOGENESIS OF HEMATOGENOUS PYELONEPHRITIS IN RATS AND ITS PRODUCTION BY A SIMPLE NEW METHOD

Abraham I. Braude, M.D., Alvin P. Shapiro, M.D. (By Invitation), and Jennie Siemienski, M.S. (By Invitation), Dallas, Tex.

Experimental pyelonephritis has usually been produced by a combination of ureteral ligation and intravenous injection of bacteria. Surgical techniques are cumbersome and the resulting disease differs from human pyelonephritis since it may occur in man without manifest hydronephrosis. In order to develop a model which would closely resemble human pyelonephritis, a nonsurgical technique was devised. It was found that light massage of rat kidneys, easily palpated through the intact body wall, consistently led to pyelonephritis after intracardiac injection of *Escherichia coli*. Renal massage without bacterial injection never caused infection or detectable renal injury.

Two groups of serial experiments were conducted in such rats: Group I received repeated cultural and microscopic examinations of urines obtained by cystotomy. Rats in Group II were sacrificed at specified periods and their kidneys and urines cultured and examined for microscopic abnormalities.

These studies disclosed that renal infection, although occasionally resulting from bacteremia alone, was markedly increased in incidence and severity by renal massage. Certain strains induced mild renal infections characterized by little or no inflammatory reaction; although *E. coli* was consistently cultured from these kidneys, it is noteworthy that urine cultures were frequently negative and pyuria almost never observed. On the other hand, when positive urine cultures were obtained, the kidneys were always infected. After terminating spontaneously in three to six weeks, these mild infections did not leave residual renal damage or render kidneys more vulnerable to repeated infection following subsequently induced bacteremia.

Other strains produced severe infections. Despite enormous numbers of *E. coli* in kidney and urine, renal inflammation and pyuria were absent in severe infections during the first twenty-four hours. By forty-eight hours inflammation began and pyuria appeared followed by extensive suppurative pyelonephritis. Hypertension or azotemia did not develop during acute pyelonephritis.

It is concluded that the slight trauma of renal massage favors development of hematogenous pyelonephritis in rats by causing large numbers of bacteria to be filtered out in the small renal vessels, that severity depends on both renal trauma and bacterial virulence, and that renal infection (proved by positive cultures of kidneys) may exist in the absence of pyuria and bacteruria.

20. HEMODYNAMIC EFFECTS OF UNILATERAL PULMONARY ARTERY OCCLUSION IN HUMAN BEINGS

BERNARD L. BROFMAN, M.D., BERNARD L. CHARMS, M.D., AND PAUL M. KOHN, M.D., CLEVELAND, OHIO

(INTRODUCED BY ERWIN LEVIN, M.D.)

By means of a newly-developed balloon catheter technique, unilateral pulmonary artery occlusion can be produced safely in human beings. Inflation of the balloon in a main branch of the pulmonary artery diverts the entire cardiac output through the other lung, thus, approximately doubling unilateral lung flow. Sudden occlusion produces *no* symptoms and *no* changes in the electrocardiogram or systemic blood pressure even in patients with severe congestive failure. In patients with normal cardiopulmonary function, unilateral occlusion produces no change in arterial oxygen saturation or cardiac output. Despite the fact that oxygen uptake occurs on only the unoccluded side and both lungs ventilate equally, total oxygen consumption and minute volume are not significantly altered. Main pulmonary artery pressure increases slightly, the degree of increase being related to the cardiac output. In patients with high cardiac output (hyperthyroids) pulmonary artery pressure shows a greater rise with unilateral occlusion.

In patients with increased pulmonary arteriolar resistance, unilateral occlusion produces a significantly greater rise in main pulmonary artery pressure. However, it can be shown that unilateral occlusion does not uniformly cause a rise in pulmonary artery pressure even in patients with elevated pulmonary artery pressures prior to occlusion. In patients with arterial desaturation as a result of pulmonary artery blood flow through poorly aerated lung, occlusion of blood flow to those areas results in increased arterial saturation and a reduction in total pulmonary resistance.

In the pulmonary artery distal to occlusion, the pressure falls to pulmonary capillary levels (as compared to simultaneously recorded wedge pressures). The blood oxygen content distal to occlusion rises to a saturation higher than that found in the brachial artery or "pulmonary capillary."

In patients being considered for lung surgery, unilateral pulmonary artery occlusion appears to be the most direct method for determining pulmonary "resection tolerance;" a marked rise in pulmonary artery pressure, a fall in cardiac output, or a fall in arterial saturation contraindicating resection of the lung under consideration.

21. RENAL EXCRETION OF ELECTROLYTES, URATE, AND PHOSPHATE IN HYPOPARATHYROIDISM

DAVID BRONSKY, M.D., ALVIN DUBIN, M.S., AND DANIEL S. KUSHNER, M.D., CHICAGO, ILL.

(INTRODUCED BY HANS POPPER, M.D.)

An observation that hyperuricemia accompanied hyperphosphatemia and oliguria in acute postoperative tetany suggested a relationship between the renal excretion of urate, phosphate, and electrolytes in the hypoparathyroid state. In three patients with acute postoperative tetany, the effect of parathyroid extract, Benemid, and dihydrotachysterol on the urinary excretion of urate, phosphate, and electrolytes was determined.

A single intravenous injection of parathyroid extract resulted in a two- to fourfold increase in the hourly excretion of water, sodium, chloride, urate, and

phosphate in two subjects. The third patient responded with phosphate diuresis alone; subsequently, she manifested resistance to therapy. No lowering of serum uric acid levels was noted.

Benemid produced in all three subjects moderately augmented urinary volumes, two- to threefold increase in excretion of sodium, chloride, urate, and phosphate, and consistent lowering of serum uric acid levels.

Dihydrotachysterol given to one patient during the recovery phase produced no change in urinary volume or phosphate, a slight but significant increase in urate, and a twofold increase in sodium and chloride excretion. With continued administration of dihydrotachysterol, serum uric acid levels remained elevated in two of the subjects.

Hyperuricemia may accompany hyperphosphatemia as a reflection of the metabolic disorder of hypoparathyroidism. Demonstration of a uricosuric effect of parathyroid extract, Benemid, and dihydrotachysterol suggests mediation by a renal mechanism. It is possible that urate and phosphate are reabsorbed by a common tubular mechanism. This is in keeping with our observation of the diuretic and phosphaturic as well as uricosuric effects of Benemid, independent of hypoparathyroidism.

22. THE LEVEL OF INFLUENZA ANTIBODIES IN SERUM POOLS DURING A SEASON OF LOW INCIDENCE

G. O. Broun, M.D., and Rose Rita Schmidt, M.S. (By Invitation)
St. Louis, Mo.

We have studied the level of antihemagglutinins and complement-fixing antibodies against various strains of influenza virus in pools made up of an average of 3,000 to 4,000 sera collected in St. Louis, Missouri, each month during the past three years. A mild Type B outbreak occurred in 1952 and an A prime epidemic of considerable proportions took place in January and February, 1953. Previously, we have reported that very low antibody levels were found against the virus strain involved in these outbreaks in each preepidemic period. A rise in antibodies against the respective virus strains followed each outbreak.

The winter of 1953 and 1954 was marked by a very low incidence of influenza infection throughout the United States. Among reported cases, Type B virus predominated.

The continued observation of serum pools indicates that the elevated antihemagglutinin levels against Type B virus present since 1952 have persisted to the summer of 1954.

The increase in A prime antibodies present since the epidemic of 1953 continued through the past winter. Antibodies against strains isolated in 1953 and the Cuppet strain FW-1-50 have in recent months returned close to preepidemic levels.

Treatment of serum pools with the receptor-destroying enzyme of *Vibrio cholerae* (R. D. E.) allows the separation of antihemagglutinin titer into "true antibody" and "nonspecific inhibitor." Changes in "true antibody" closely parallel the curves of total antihemagglutinin titer. "Nonspecific inhibitor" as well as "true antibody" increased in the serum pools following the influenza outbreaks. This may mean that increase in nonspecific inhibitor is a part of the immunological reaction to exposure.

A rise in complement-fixing antibodies is noted following influenza outbreaks, but this elevation is less persistent than is the case with antihemagglutinins. Continued experience with the use of serum pools supports the impression that these serve as a useful tool for estimating the level of immunity

of a population against prevalent strains of influenza virus. The level of antibodies against the 1953 A prime strains has fallen to levels which suggest the possibility of a recurrence of infection with these viruses during the coming winter.

23. THE SIMULTANEOUS TIME COURSE CURVES OF VOLUMES, RATES, AND ACCELERATION IN RATES OF DIGITAL INFLOW AND OUTFLOW DURING A SINGLE PULSE CYCLE IN MAN WITH AORTIC INSUFFICIENCY

G. E. Burch, M.D., New Orleans, La.

Simultaneous time course curves of volumes, rates, and acceleration in rates of inflow and outflow of the finger tip during a single pulse cycle were recorded for eight patients with aortic insufficiency and 25 controls by means of a newly developed venous occlusive pneumoplethysmographic method. These curves assisted in the elucidation of digital arterial and venous aspects of peripheral vascular adjustments in aortic insufficiency (A.I.).

The curves in A.I. differed considerably from normal; for example, maximal rates of inflow in A.I. exceeded those in normal man. Because the method also permits, for the first time, recording simultaneously basal rates of inflow and outflow, the low total flow observed was attributable to low basal flow.

Inflow rate rapidly increased and decreased during early systolic phase of pulse cycle, ending well before onset of the diastolic phase, thus defining the time course of capillary pulse in A.I. The curves suggest loose arterial, arteriolar, capillary, A-V anastomotic vessels and venules in A.I. Digital venous tone was increased in several patients due probably to mild subclinical congestive failure.

The time course of volume ejection and work output of the left ventricle contributed much to configuration of the curves, but peripheral vascular factors were important. Since digital curves reflected energy and volume output of the left ventricle, work and power developed by the ventricle in A.I. was high.

The wide spread in time and magnitude of the inflow and outflow curves clearly revealed a distensible digital vascular bed, with possible exception of digital veins in some patients. As revealed by inflow and outflow curves with the finger 45 cm. above heart level, elevation further decreased tightness of the vascular bed and reduced "leakage" past the occluding cuff, but failed to displace temporally the systolic surge of inflow in A.I. Elevation decreased digital inflow in the normal subjects and several patients with A.I.

The curves in A.I. differed significantly from normal man with marked peripheral dilatation produced by hot and humid environment. The simultaneous curves varied considerably among patients and, from time to time, in the same patient and revealed interesting temporal quantitative and qualitative hemodynamic phenomena.

24. UNUSUAL R-T SEGMENT DEVIATIONS IN ELECTROCARDIOGRAMS OF NORMAL PERSONS

Howard B. Burchell, M.D., L. Guy Chelton, M.D. (By Invitation), Rochester, Minn.

Normal electrocardiograms characterized by absence of any isoelectric period between the QRS and the succeeding concordant T deflection are unusual when the resulting S-T segment shift exceeds 0.1 mv. The electro-

cardiographic feature noted is usually an elevation of the R-T segment and acute pericarditis, myocardial ischemia or even instrumental aberration may be suspected.

This study concerns the clinical and electrocardiographic analysis of 100 patients having tracings which showed elevation of the R-T segment of 1 mm. or more (0.1 mv.) in standard or left precordial leads.

The patients followed the total group referred to the electrocardiographic laboratory in regard to age and race, but all but one were men and all were normotensive. In approximately 50 per cent of the group studied the primary diagnosis was anxiety state with "functional" cardiac symptoms; in about 30 per cent the elevation of the R-T segment was incidental in the examination, in about 10 per cent minor valuvular diseases were present, and in 10 per cent a mild coronary insufficiency syndrome was diagnosed. None had cardiac enlargement as judged by the teleoroentgenogram.

The electrocardiographic pattern was repetitive, being constant from day to day, and in 16 instances for which we had records, obtained more than a year apart, it persisted. The various electrocardiographic periods, that is, RR, PR, QRS, and QT, were within the normal range. Exercise tests were carried out on 17 patients with uniform results, the R-T segment becoming isoelectric but returning to its pre-exercise level within 3 minutes. Esophageal electrocardiograms showed S-T depression at the atrial level and R-T elevation at the ventricular level.

Atropine, methacholine, and hydergine in usual therapeutic doses had no effect on the electrocardiographic pattern. The spatial vector for the R-T segment (or the persistent systolic potential) was constructed and was oriented downward and forward, and in the horizontal plane tended to point more laterally in hearts of the "electrocardiographic horizontal" type than in the "vertical type."

25. THE EFFECT OF CORTICOTROPIN (ACTH) ON CALCIUM OUTPUT IN DOG BILE

WILLIAM BURNETT, M.B., F.R.C.S. (BY INVITATION), AND
EDMUND B. FLINK, M.D., MINNEAPOLIS, MINN.

A negative calcium balance with resultant osteoporosis occurs in Cushing's syndrome and during prolonged ACTH or cortisone therapy. Some balance studies have indicated that the excessive loss of calcium occurs in the stool.

In order to test a theory that calcium is excreted by the liver during ACTH administration, four dogs had bile-renal fistulas formed. Control experiments using four normal dogs were also carried out. Urine was collected for two three-day periods before, during, and after ACTH. The average urinary output of calcium during control period was 0.54 mg./kg./day in normal dogs and was 1.63 mg./kg./day in the bile renal fistula dogs.

Therefore, there normally is some excretion of calcium in bile. The urinary output of calcium was not significantly altered in the normal dogs by ACTH administration; but it was increased in every dog with bile-renal fistula, and the increase persisted for at least one three-day period after ACTH was discontinued. The average increase for the group was 17.5 mg./day during ACTH administration. This is a 75 per cent increase over control period. On the basis of these data a possible explanation of the increased excretion of calcium in the feces during adrenal cortical hyperfunction or hormone excess can be made.

26. HEMODYNAMIC STUDIES BEFORE, DURING, AND AFTER OPERATION IN 10 CASES OF PATENT DUCTUS ARTERIOSUS WITH EQUIVALENT PULMONARY AND SYSTEMIC ARTERIAL PRESSURES

JOHN A. CALLAHAN, M.D. (BY INVITATION), JOHN W. KIRKLIN, M.D. (BY INVITATION), HOWARD B. BURCHELL, M.D., AND EARL H. WOOD, M.D., PH.D., ROCHESTER, MINN.

The management of patients with patent ductus arteriosus and severe pulmonary hypertension remains controversial, and the additional association of a veno-arterial shunt through the ductus is generally believed to contraindicate operation.

Hemodynamic studies are reported in 10 of the 29 cases of patent ductus and pulmonary hypertension treated surgically at the Mayo Clinic. In these 10 cases the pulmonary and systemic arterial pressures were equivalent. In 7 of the 10 an attempt was made to detect reversal of flow through the ductus by comparing radial and femoral arterial oxygen saturations, and, in 4, a right-to-left shunt was demonstrated.

All patients were studied by cardiac catheterization preoperatively and 7 were studied during operation. Pressures from the pulmonary and systemic arterial systems, arterial dye-dilution curves, and arterial oxygen saturations were recorded before, during, and after permanent division of the ductus arteriosus. Four of the patients were studied by cardiac catheterization 4 months to 2 years postoperatively.

Age of patients averaged 8 years (range: 10 weeks to 22 years). Pulmonary artery pressure, preoperatively, averaged 92 mm. Hg (range: 69 to 118 mm. Hg).

In 5 of the 7 patients studied during operation it was demonstrated that the predominant shunt through the ductus arteriosus was left to right since temporary occlusion of the ductus by clamping produced an immediate fall in pulmonary artery systolic pressure, averaging 27 mm. Hg (range: 15 to 50), and a rise in systemic arterial pressure, averaging 28 mm. Hg. In 2 patients large right-to-left shunts existed which were demonstrated by arterial desaturation and abolished by closure of the ductus. Occlusion of the ductus increased pulmonary artery pressure by 7 and 25 mm. Hg and decreased systemic arterial pressure by 8 and 23 mm. Hg, respectively. Dye-dilution curves confirmed the presence of these shunts and their elimination by closure of the ductus.

In the 4 patients studied postoperatively the pulmonary artery systolic pressure had decreased by an average of 47 mm. Hg (range: 11 to 77 mm. Hg). In 1 of the 4, closure of the ductus at operation had increased pulmonary artery pressure.

27. GROSS HEMATURIA IN SICKLE CELL TRAIT AND SICKLE CELL C-HEMOGLOBIN DISEASE

A. ZERNE CHAPMAN, M.D. (BY INVITATION), PAUL S. REEDER, M.D. (BY INVITATION), IRVING A. FRIEDMAN, M.D. (BY INVITATION), AND LYLE A. BAKER, M.D., HINES, ILL.

Hematuria in sickle cell anemia has been previously recorded in the literature. Congestion of small vessels, thrombosis, stasis, and infarction necrosis in the kidneys have been described, associated with capillary bed blockage

with sickled red cells between the collecting tubules. The incidence of hematuria in sickle cell anemia as reported by others has ranged from 3 in 54 cases to 2 in 142 cases.

Several recent reports in the literature have indicated a significant incidence of hematuria in patients with sickle cell trait. It is possible that certain of the cases previously reported were instances of sickle cell C-hemoglobin disease.

The present study concerns eight cases of "idiopathic" gross hematuria occurring in Negro patients who were seen at the U. S. Veterans Administration Hospital, Hines, Illinois, and Cook County Hospital, Chicago, Illinois. They were thoroughly evaluated by genitourinary and hematological studies, including filter paper electrophoresis and fetal hemoglobin determinations. Five of the patients were sickle cell traits and three were sickle cell C-hemoglobin disease. Two of the patients had had nephrectomies performed and kidney sections revealed sickling of red blood cells in the vessel lumina and glomeruli, with submucosal hyperemia, necrosis, and evidence of hemorrhage. The etiological aspects of the disease are discussed with reference to the inherent susceptibility of the kidney to local infection, ischemia, and other forms of stress associated with the sickling properties of the red blood cells in this disease.

Sickle cell trait is generally considered to be relatively benign, and since sickle cell C-hemoglobin disease is more related in clinical course to sickle cell anemia, it was expected that more of our cases would be sickle cell C-hemoglobin in type. However, the incidence of hematuria in sickle cell trait in this and other studies is felt to be significant and necessitates a re-evaluation of our concepts concerning the benignity of this process.

28. ELECTROCARDIOGRAPHIC OBSERVATIONS DURING QUINIDINE ADMINISTRATION TO PATIENTS WITH ATRIAL FIBRILLATION

I. Pseudo-Prolongation of Q-T Interval Due to Merging of T-Wave With U-Wave

John Cheng, M.D. (By Invitation), George C. Sutton, M.D. (By Invitation), William P. Swisher, M.D. (By Invitation), and Don C. Sutton, M.D., Chicago, Ill.

Detailed analyses of the electrocardiograms were made in patients with chronic atrial fibrillation during treatment with quinidine sulfate in an attempt to convert the abnormal rhythm to sinus rhythm. Ninety-two patients with 125 attempts at conversion with quinidine were included in this study. Daily plasma levels of quinidine were determined and electrocardiographic recordings of the three standard limb leads and three unipolar precordial leads, V_1, V_2, and V_4, were taken at the same time daily or several times a day, if and when important changes were observed or expected. The various components of the electrocardiograms were measured and compared with normal values for a given heart rate. The measurements were again correlated with the levels of plasma quinidine.

The typical pattern at the peak level of quinidine is characterized by a depression of the S-T segment, depression or inversion of the T wave, and appearance or exaggeration of the U wave with fusion with the descending limb of the T wave in the mid-precordial leads. This pattern, which was found in most of our cases, resulted in a pseudo-prolongation of the Q-T interval. As the level of plasma quinidine declines, the S-T segment is less depressed,

the T waves less inverted or more upright and the U waves diminish in amplitude and become separate from the T waves. In our cases that showed these discordant changes of the T and U waves, the Q-T intervals are essentially unchanged, although in some cases a true prolongation of Q-T interval with or without a notched T wave also occur. This study is being continued.

29. NORTH AMERICAN BLASTOMYCOSIS

A Clinical and Therapeutic Study of Forty Cases

Edward I. Cherniss, M.D., Milwaukee, Wis.

(Introduced by Burton A. Waisbren, M.D.)

The recent introduction of the diamidines in the therapy of North American blastomycosis focused our attention on the natural history of this disease. The records of forty cases of North American blastomycosis including thirty-six patients with systemic involvement were studied. Age, sex, race, nativity, residence, occupation, season of onset, history of trauma, history of respiratory infection, presenting complaints, clinical features, extent of involvement, therapy, and prognosis were analyzed. In addition to the pertinent data regarding these factors, three cases treated with stilbamidine will be presented in detail. In each of these cases a gratifying clinical response was obtained.

In no instance could the source of the disease, its means of transmission or mode of infection be demonstrated. No evidence suggested human transmission or animal source. No significant relation was apparent to the occupational exposures of the patient, and there was no preponderant season of onset. The previously observed frequency of infection in the adult male and its tendency to favor the 40- to 60-year age group was again demonstrated. No particular racial susceptibility was found. A diversity of presenting symptoms and clinical patterns reflected the multiplicity of systems affected. Organs were involved in the following frequency: skin, subcutaneous tissue, lung, and bone.

Spontaneous recovery or remission did not occur. Improvement short of complete recovery was minimal and short lived in most instances. The majority of fatalities occurred within four years of the onset of the disease. Those who survived seven years appeared to have been cured. Thus a standard by which to judge the ultimate efficacy of therapy was indicated.

30. CLINICAL, HEMATOLOGICAL, AND GENETIC STUDIES OF HEMOGLOBIN E

Amoz I. Chernoff, M.D., Virginia Minnich, M.S. (By Invitation), Soodsakorn Chongcharoensook, M.D. (By Invitation), Supa NaNakorn, M.D. (By Invitation), and Renate Chernoff, M.S. (By Invitation), St. Louis, Mo.

The clinical, hematological, and genetic effects of hemoglobin E have been investigated in a large number of Thai individuals. Hemoglobin E is a newly described abnormal hemoglobin which has an electrophoretic mobility intermediate between hemoglobin C and hemoglobin S. This report is based on a study of twenty subjects with hemoglobin E trait, five with pure hemoglobin E disease, and 14 with Mediterranean-hemoglobin E disease.

Individuals with hemoglobin E trait were asymptomatic. Hematological values were within normal limits except when other disease processes were implicated. The hemolytic index, osmotic fragility, and target cell counts

showed no specific abnormalities. Paper eelctrophoresis of hemoglobin solutions from individuals with hemoglobin E trait revealed two components with the mobilities of hemoglobin A and hemoglobin E. By alkali denaturation, normal amounts of fetal hemoglobin were found.

Among the five cases of pure hemoglobin E disease, splenomegaly was found in three and hepatomegaly in two. All complained of tiredness, easy fatigability, arthralgias, and two of icterus. Hematological studies showed strikingly microcytic, normochromic erythrocytes, normal erythrocyte levels, no increase in reticulocytes, 25 to 60 per cent target cells, slight erythrocytic hyperplasia of the marrow, and a marked symmetrical shift to the right of the osmotic fragility curve. The hemolytic index in one patient was normal. Hemoglobin analysis revealed from 94 to 98 per cent hemoglobin E and from 2 to 6 per cent hemoglobin F.

The fourteen patients with Mediterranean-hemoglobin E disease had a severe hemolytic anemia almost indistinguishable from Cooley's anemia. Clinically, their course was usually somewhat milder so that the majority had lived to adulthood. They required fewer transfusions, lacked definite Mongoloid facies, and appeared less cachectic. In several, splenectomy had apparently partially alleviated the hemolytic process. Hematological changes were those found in Cooley's anemia although hypochromia was somewhat less marked. From 5 to 20 per cent of erythrocytes were target cells. The osmotic fragility curve was asymmetrically shifted to the right. Reticulocyte counts, bone marrow sutdies, and hemolytic indices were compatible with a severe hemolytic process. Two hemoglobin components were present, hemoglobin E and hemoglobin F. Hemoglobin E comprised from 60 to 80 per cent of the total.

Genetic studies of families having the gene for hemoglobin E indicated the hereditary nature of this newly observed abnormality of hemoglobin synthesis.

31. CORRELATED OBSERVATIONS ON HEPATIC HEMODYNAMICS, CHEMICAL LIVER FUNCTION TESTS, AND HISTOPATHOLOGIC CHANGES IN PATIENTS WITH REPRESENTATIVE LIVER DISEASES

James A. Clifton, M.D. (By Invitation), George H. M. Thornton, M.B. (By Invitation), John W. Eckstein, M.D. (By Invitation), and James W. Culbertson, M.D., Iowa City, Iowa

Fourteen patients with portal cirrhosis in fatty and fibrotic stages, cholangitis, infectious hepatitis, or sarcoid were studied with the usual liver function tests, liver biopsy, and hepatic vein catheterization. Determinations of occluded distal hepatic vein pressure (ODHVP), estimated hepatic blood flow (EHBF), plasma clearance (Cbsp) and hepatocellular extraction (Ebsp) of bromsulphalein were made. Adequate biopsy specimens were available in ten of these patients.

As has been demonstrated by others, the alterations in the usual chemical liver function tests in this group of patients were found to parallel changes in the histologic picture only in a general way. Similarly, the EHBF failed to correlate with the degree of histologic abnormality. In contrast to this, the Cbsp and the Ebsp varied directly with the pathologic picture. Results of the standard clinical BSP retention test paralleled more closely the Cbsp and the histopathologic appearance than did the values of the other usual chemical tests. Cbsp is influenced by two factors, hepatic blood flow rate and hepato-

cellular extraction fraction. In these patients the mean EHBF was normal, while the mean Ebsp was low, resulting in a low mean Cbsp.

It is of interest that the ODHVP correlated better with morphologic change than did the EHBF. Four patients had elevated ODHVP without esophageal varices. In two of these the ODHVP fell to normal levels following conventional hospital therapy of their cirrhosis, and the EHBF rose significantly, although there was no consistent relationship in the whole group between EHBF and ODHVP. No significant improvement was noted in repeat liver biopsies from these patients. The possible relationship of this falling ODHVP to the phenomenon of "disappearing" esophageal varices is under investigation in our laboratory.

It appears that adequate hepatic blood supply may be maintained despite moderate impairment of hepatocellular structure and function. Hepatocellular abnormality is reflected better by standard and refined bromsulphalein methods than by other clinical tests. Elevated portal venous pressure and slow hepatic blood flow may be improved by treatment of cirrhosis.

32. CORRELATIONS BETWEEN PLASMA PROTEIN FRACTIONS, ANTIBODY TITERS, AND THE PASSIVE TRANSFER OF DELAYED AND IMMEDIATE CUTANEOUS REACTIVITY TO TUBERCULIN PPD AND TUBERCULOPOLYSACCHARIDES

LEON R. COLE, M.D., AND CUTTING B. FAVOUR, M.D., BOSTON, MASS.

(INTRODUCED BY GUSTAVE J. DAMMIN, M.D.)

Guinea pig donors were immunized with heat-killed tubercle bacilli suspended in mineral oil. Large volumes of plasma, obtained from these donors 24 to 48 hours after a tuberculin skin test, were fractionated by Cohn's Method X (J. Am. Chem. Soc. 72: 465, 1950), and the fractions were injected into normal animals intraperitoneally.

Two chemically distinct antibodies were identified.

a. Antibody to tuberculopolysaccharide, measured by the Middlebrook-Dubos test (J. Exper. Med. 88: 521, 1948), is contained exclusively in fraction II (gamma globulin). It passively transfers anaphylaxis and urticarial-type skin reactivity to tuberculopolysaccharide.

b. Antibody to tuberculoprotein, measured by the Boyden technique (J. Exper. Med. 93: 107, 1951), is contained exclusively in a new plasma fraction which we have defined and named fraction IV-10. By standard Cohn's Method X, Fraction IV-10 falls into Fraction IV (alpha globulin) and to a lesser extent V (albumin). Fraction IV-10 is initially soluble in 19 per cent alcohol at pH 6.0, temperature −5° C., but precipitates overnight. This property permits its initial separation from I, II, and III, and later isolation from IV and V. It represents about 0.6 per cent of the plasma protein. Fraction IV-10 passively transfers a delayed-type skin sensitivity to tuberculin PPD. The skin reaction begins 3 to 4 hours after application of PPD and is maximal at 18 to 24 hours. Fractions IV + IV-10 and IV + V + IV-10 are also capable of passive transfer of the delayed reaction. A period of 3 to 6 days usually occurs from the time of intraperitoneal injection to onset of skin reactivity. Addition of PPD to the fraction, or adsorption with Boyden-sensitized cells, results in failure of transfer. When fractions II and IV-10 are combined the antibody to tuberculopolysaccharide completely inhibits the delayed-type skin reactivity.

Fraction IV-10 is heat labile. This property was demonstrated in the serum or plasma of eighteen sensitized guinea pigs, tested by the Boyden method. All eighteen aliquots in which complement was inactivated with

sodium ethylenediamine tetracetate gave significant titers. Aliquots of the 18 specimens in which complement was inactivated at 56° C. for 30 minutes gave the following results: 6 were negative, 2 were not significant, and the titers of the remaining 10 were strikingly lower than their corresponding EDTA-inactivated aliquots.

33. INTRAPROSTATIC INJECTION OF RADIOACTIVE YTTRIUM CHLORIDE IN THE DOG

JOHN A. D. COOPER, M.D., PH.D., GEORGE J. BULKLEY, M.D. (BY INVITATION), AND VINCENT J. O'CONOR, M.D. (BY INVITATION), CHICAGO, ILL.

Following the intraprostatic injection of yttrium^{90} chloride in the dog, approximately one-half of the activity was excreted in the urine in 96 hours. The major portion appeared in the first 48 hours. One-third of the activity remaining in the body was in the prostate gland and only 6 per cent in the liver. Only small amounts of radioyttrium were found in the spleen, lung, and bone marrow. Significant activities were found in regional lymph nodes. After removal of the bladder, prostate, urethra, and identifiable lymph nodes a large amount of radioactivity was found to be distributed through the pelvic tissues, where it apparently had been carried by direct extension from the prostate along fascial planes or through lymphatic channels.

Animals were sacrificed at intervals up to three months to obtain tissues for histologic study. The acute radiation reaction in the prostate was characterized by hemorrhage, exudation, and necrosis. Healing began in five to six weeks with replacement of necrotic areas with fibrous connective tissue, decrease in the number of glands, and reduction in size of the prostate and was essentially complete in three months. Lymph nodes in which radioactivity was present showed hemorrhage, decrease in the number of follicles, some central necrosis and pigmentation. There were no histologic changes in the distant organs which could be attributed to radiation effects.

Results in the dog suggest that radioyttrium may offer advantages over radiogold and radioactive chromic phosphate in the treatment of carcinoma of the prostate.

34. THE CONCEPT OF RENAL Q_{O_2}/Tm: APPLICATION TO THE STUDY OF RENAL DISEASE

ARCHER P. CROSLEY, JR., M.D., GEORGE G. ROWE, M.D., JOHN H. HUSTON, M.D., AND LEE L. SCHLOESSER, M.D., MADISON, WIS.

(INTRODUCED BY WILLIAM S. MIDDLETON, M.D.)

Previous investigations of renal oxygen consumption (Q_{O_2}) (total renal blood flow × arterial-renal venous oxygen difference) have assumed a relationship between this factor and renal hemodynamic measurements, constituents of surface area, or an estimated renal weight. Since it seemed more reasonable to presume that oxygen utilization was related to the functional tubular mass, the latter was determined simultaneously as the tubular secretory capacity of PAH (Tm_{PAH}) in 5 normotensive subjects, 5 hypertensive patients, 5 patients with diabetic nephropathy, 2 patients with pyelonephritis, and one labile diabetic individual without evidence of renal disease.

The results indicate that the ratio Q_{O_2}/Tm is not significantly different in normotensive and hypertensive patients. Individuals with diabetic nephropathy, however, showed an elevation of this factor. A comparison of the data between groups would suggest that such an enhancement may be related to an active intrarenal inflammatory process.

35. THE HYPERTONIC CONCENTRATIONS OF SODIUM AND POTASSIUM IN THE CONTENTS OF THE TERMINAL ILEUM DURING THE ADMINISTRATION OF A CATION-EXCHANGE RESIN

R. E. Dailey, M.S. (By Invitation), Leon Swell, Ph.D. (By Invitation), E. C. Trout, Jr., B.S. (By Invitation), and H. Field, Jr., M.D., Martinsburg, West Va.

During the studies of the electrolyte changes in different parts of the gastrointestinal tract, with and without the simultaneous administration of a carboxylic cation-exchange resin, there was observed an unexpected finding. During the administration of the resin, there was an increase in the unbound sodium and potassium in the contents of the terminal ileum, producing strongly hypertonic solutions. Those increases were not explained by the composition of the contents of the jejunum, which had lower concentrations of both free and bound sodium and potassium, during such treatment.

The findings in the terminal ileum were reproduced, in vitro, by exposing ileal contents to resin which had previously been exposed to much higher concentrations of sodium and potassium, at the pH of the ileal sample used. The result of that exposure was a release of both sodium and potassium to the ileal fluid. Simultaneously, there was a decrease of 0.3 unit in the pH of that fluid.

The only ions which could exchange with sodium and potassium, on the resin, and, at the same time, acidify the solution are hydrogen ions. Such ions would need to be produced; de novo, from the ileal contents, in the test tube.

It seemed possible that sufficient carbonic anhydrase might have previously diffused from cells so as to be capable of producing hydrogen ions, probably with the coactivity of enzyme systems in the living cells which should be present in the contents of the terminal ileum. In a few experiments, in which equal aliquots of the ileal contents were heated to 65° C. for one-half hour, to destroy all enzymes, prior to exposure to the resin, there was a considerable release of sodium and potassium to the ileal fluid, but less than was released from the resin in the previously unheated ileal contents.

These findings also suggest that, during the passage from the jejunum to the ileum, additional amounts of sodium and potassium were bound by the resin and that, in the terminal ileum, those ions were being released from the resin faster than they were being absorbed.

36. RENAL HOMOTRANSPLANTATION IN MAN: CASE WITH UNUSUALLY LONG FUNCTIONAL SURVIVAL

Gustave J. Dammin, M.D., David M. Hume, M.D. (By Invitation), John P. Merrill, M.D. (By Invitation), Benjamin F. Miller, M.D., and George W. Thorn, M.D. (By Invitation), Boston, Mass.

A major difference between renal homotransplants in dog and man is the duration of functional survival of the homotransplant. In the dog, the average duration is about five days with an upper limit of about three weeks. In man, average duration cannot be estimated because of the relatively small number of homotransplants performed, but in the following case, functional survival lasted almost six months.

The patient, G. W., was a 26-year-old physician with terminal chronic glomerulonephritis. The homotransplant, from an adult patient who died during a cardiac operation, was inserted into a polyethylene sac and placed subcu-

taneously into the thigh with vascular anastomoses to the femoral vessels and with a cutaneous ureteral orifice. Significant function appeared on the nineteenth postoperative day and for five months the homotransplant excreted between 1,500 to 2,000 ml. of urine per day. During this period, the patient's condition improved and the blood urea nitrogen dropped to 33 mg. per cent from a postoperative level of 244 mg. per cent. The blood pressure remained elevated (220/140), congestive failure appeared, and the patient expired six months postoperatively.

Post mortem, the transplanted kidney weighed 340 grams. Vascular anastomoses were intact and endothelialized. The ureter was slightly dilated and angulated. There was no pyelonephritis. The host epidermis had grown over the cutaneous portion of the ureter. The ureter was well vascularized and its smooth muscle intact. No cellular reaction was observed at the anastomotic junctions of host and transplant tissues. Since the other donor kidney was essentially normal, the advanced atherosclerosis with luminal narrowing observed in the smaller branches of the renal artery of the transplant must have developed during its tenure in the recipient.

Microscopically, the glomeruli were normal except for moderate basement membrane thickening. There was advanced atrophy of the tubules particularly in the cortex. However, some tubules appeared normal or hypertrophied. There was advanced interstitial edema and moderate lymphocytic infiltration with some mononuclear cells showing pyroninophilia.

The long survival and morphologic changes observed suggest considerable compatibility of recipient and donor tissues and that ultimate failure of the transplant resulted from vascular and circulatory changes related to hypertension and decompensation.

37. THE RELATION OF ANTIBODY CONTENT IN THE POPULATION TO RECENT EXPERIENCES WITH INFLUENZA VIRUSES IN ENGLAND AND THE UNITED STATES

Fred M. Davenport, M.D., Ann Arbor, Mich.

In 1951, a severe epidemic of influenza A-prime occurred in England. Yet in the same year the outbreak experienced in the United States was mild even though virus of similar antigenic constitution was widely disseminated in both countries. The greater severity of the epidemic in England may have been due in part to a lower level of immunity in the British population because of infrequent exposures to influenza viruses in recent years. A previous study demonstrated that a serologic recapitulation of the past experiences of a population with influenza could be obtained by determining the age distribution of antibody against many strains of virus, and that the character of antibody present at several periods of life correlated with the immunity of each age group. The objective of the present investigation was to compare the serologic records of past infection in the British and American populations, and to determine whether the results reflected differences in the recent epidemic experience of the two countries. For this purpose sera were collected in 1953 in Ann Arbor, Michigan, and Sheffield, England, pooled by 2- to 5-year age groups, and tested for hemagglutination-inhibiting antibodies after removal of nonspecific inhibitors by digestion with trypsin. Sera from Sheffield were made available through the generosity of Dr. Stuart-Harris. Levels of antibody against A and A-prime strains of influenza virus were markedly lower in the pools of sera collected in Sheffield, although the age distribution of maximal antibody titer was approximately the same in both collections. With several A-prime strains, antibody could not be detected in the Sheffield pools, although the Ann Arbor pools contained moderate levels of

antibody against the same viruses. The low levels of strain specific antibody in the early years of life and of composite antibody in adulthood found in the Sheffield sera helps to explain the high attack rate of influenza in England in 1951 and suggests a reason for the unusual finding that the attack rate of influenza A-prime appeared to be the same at all ages in that outbreak.

38. STUDIES ON PORTAL PRESSURE

The Relation of Intrasplenic Pulp Pressure to Portal Venous Pressure, and the Use of Splenic Explant in the Study of Portal Venous Pressure

W. D. Davis, Jr., M.D., Hugh M. Batson, Jr., M.D. (By Invitation), and William Schindel, M.D. (By Invitation), New Orleans, La.

The intimate relationship of the sinusoids of the splenic pulp to the portal venous system suggests that intrasplenic pulp pressure might precisely reflect the level of portal venous pressure. Direct measurement of intrasplenic pulp pressure is easily accomplished by means of the Burch phlebomanometer and tissue pressure needle with side openings and closed end, using sodium citrate as anticoagulant. Preliminary acute experiments in dogs demonstrated that occlusion of the splenic vein by ligature was followed by rapid rise of the intrasplenic pressure to approximately 450 mm. of water with a rapid fall to normal following release. Simultaneous recordings of portal venous pressure by means of a direct water manometer and intrasplenic pulp pressure demonstrated close agreement between the values obtained under control conditions, when portal hypertension was acutely induced by occlusion of the portal vein, and rapid and simultaneous fall following release.

The preparation of dogs with subcutaneous splenic explants has provided a method by which variations in portal pressure may be followed in the unanesthetized animal serially for periods of months or years. Previously described acute elevation of the portal pressure in anesthetized animals from epinephrine, norepinephrine, and histamine has been demonstrated by this technique, as well as a slight fall from surgical pituitrin. Following constriction of the portal vein with an aluminum band the splenic pulp pressure rises rapidly to a peak of 250 to 300 mm. in H_2O, remains stabilized for about 5 to 7 days, then slowly falls to the preoperative level. During this period of portal hypertension the rise in splenic pulp pressure from epinephrine is unchanged but surgical pituitrin administration invariably has reduced the intrasplenic pressure to the preoperative level or below for periods as long as 30 minutes. This depressor effect is also exhibited by pitressin but to a much lesser degree of pitocin. Investigation of the mechanism of this effect and of the effect of epinephrine and pituitrin in hypertension secondary to hepatic vein constriction is continuing.

39. THE EFFECTS OF FRUCTOSE ON MYOCARDIAL METABOLISM

Valentino DeFazio, M.D. (By Invitation), Harper K. Hellems, M.D., Leonard Leight, M.D. (By Invitation), Frederick N. Talmers, M.D. (By Invitation), and Timothy J. Regan, M.D. (By Invitation), Detroit, Mich.

To determine if fructose is utilized by the myocardium, six normal fasting subjects have been studied by the technique of coronary sinus catheterization during the intravenous infusion of 10 per cent fructose solution at the rate

of 1.5 to 2.0 Gm./kilogram/hour. Simultaneous arterial and coronary sinus blood samples were obtained before, at 15 minutes, and 57 minutes after starting the infusion, and in three patients 15 minutes after completion of the infusion. All samples were analyzed for total reducing substances, fructose, lactate, and pyruvate. In four of the subjects, the myocardial respiratory quotient was determined before and during the infusion of fructose.

During the infusion of fructose, arterial blood levels varied from 22 to 291 mg. per cent, while myocardial extraction varied from 4.6 to 19.3 mg. per cent in four subjects, and was less than 3 mg. per cent in two individuals. There was no relation between myocardial extraction and blood fructose concentration. After cessation of the infusion, negative myocardial A-V differences of fructose varying from 2 mg. to 14.3 mg. per cent were observed, suggesting that fructose may be trapped in the myocardial cell and then released in a free form.

In all subjects during the infusion of fructose, there was a marked increase in arterial blood concentrations of lactate and pyruvate and of their extraction by the myocardium. Although a consistent linear relationship could not be demonstrated between arterial concentration and myocardial extraction of these substances, the greater extractions tended to be associated with the higher blood concentrations. In four of the six subjects in whom the myocardial respiratory quotient was determined, the control value averaged 0.79 and increased to 1.03 during fructose infusion.

It is concluded that these observations, while demonstrating the myocardial cellular extraction of some fructose, do not afford evidence that the hexose is utilized by the cell for energy metabolism.

40. TYPE-SPECIFIC STREPTOCOCCAL ANTIBODIES

Floyd W. Denny, Jr., M.D., William D. Perry, M.D. (By Invitation), Lewis W. Wannamaker, M.D. (By Invitation), and Charles H. Rammelkamp, Jr., M.D., Nashville, Tenn.

Type-specific or M-protein antibodies have been shown to be responsible for immunity of man to streptococcal infections, but little is known of the behavior of these antibodies. This study was designed to determine the frequency and time of development, with relation to serologic types, of type-specific antibody and antistreptolysin "O." Patients with streptococcal infections who received either penicillin or Aureomycin therapy were also studied, as were those with streptococcal infections who subsequently developed acute rheumatic fever.

Ninety-two per cent of untreated patients showed a significant increase of type-specific antibody and 98 per cent a significant increase of antistreptolysin. Antistreptolysin developed quite rapidly, appearing in most cases within 30 days after infection. No difference in time of development due to the infecting type of streptococcus could be demonstrated. In contrast, type-specific antibodies developed much later and there was a great difference in the time of response according to the type of infecting streptococcus. Patients with Type 6 or 24 infections developed type-specific antibodies most rapidly, the majority showing significant increases within 40 to 50 days. Patients with Type 5 or 14 infections developed antibodies more slowly, very few showing significant increases until after 50 days.

Treatment with penicillin caused a moderate reduction in the antistreptolysin response and completely inhibited the production of type-specific

antibodies. Throat cultures following penicillin therapy were uniformly negative for streptococci. Treatment with Aureomycin caused little if any reduction in the antistreptolysin response. Type-specific antibodies were moderately reduced but not to the degree caused by penicillin. The failure of Aureomycin to prevent formation of type-specific antibody was closely related to its failure to eradicate the streptococcus from the throat.

The type-specific antibody pattern of those patients who subsequently developed rheumatic fever was not significantly different from the control group.

These data emphasize the differences in host response to various antigenic components of Group A streptococci. They also emphasize the effectiveness of penicillin in treatment of streptococcal infections and have certain implications concerning the pathogenesis of rheumatic fever.

41. STUDIES OF THE ACUTE HEMOLYTIC ANEMIA INDUCED BY CERTAIN ANILINE DERIVATIVES AND RELATED COMPOUNDS

RAYMOND J. DERN, M.D., PH.D. (BY INVITATION), ERNEST BEUTLER, M.D. (BY INVITATION), C. LARKIN FLANAGAN, M.D. (BY INVITATION), AND ALF S. ALVING, M.D., CHICAGO, ILL.

It has long been recognized that methemoglobinemia and acute hemolytic anemia are two of the important toxic effects resulting from the administration of certain aniline derivatives and related compounds. Many studies of drug-induced methemoglobinemia have been made, with the accumulation of considerable evidence that there is no cause-effect relationship between methemoglobin formation and acute hemolysis. A detailed study of the mechanism of the hemolytic anemia produced by aniline derivatives has been undertaken in this laboratory.

It was first shown that an erythrocytic defect, present in about 15 per cent of American Negroes and in occasional Caucasians, was responsible for the development of acute hemolytic anemia when one of these drugs (primaquine) was given. Subsequent studies demonstrated that the presence of this defect also causes sensitivity to sulfanilamide, acetanilid, phenylhydrazine, thiazolsulfone (Promizole), phenacetin, and sulfoxone (Diasone).

The administration of these compounds to sensitive men results in acute hemolysis, followed by a return of hemoglobin concentration to normal levels, although drug administration is continued. The development of a drug refractory state is due to the fact that a sensitive individual has a heterogeneous cell population, with old cells sensitive and young cells refractory to drug. Therefore, the administration of drug results in hemolysis of old cells only, the hemoglobin returning to normal by virtue of the rapid production of new cells which are not affected by drug until they age in the circulation.

Although sensitive erythrocytes are normal morphologically, in response to fragility tests, and in hemoglobin type, they differ from nonsensitive cells in two respects. When sensitive red cells are incubated with acetylphenylhydrazine and subsequently stained for microscopic examination, a characteristic pattern of Heinz body formation develops. This has been made the basis of a highly reliable in vitro test for sensitivity. Biochemical differentiation is also possible since it has been shown that sensitive red cells have a consistently lower glutathione level than nonsensitive cells.

42. PROLONGED BALANCE STUDIES OF SODIUM^{22} AND ITS RELATION TO SODIUM INTAKE

WM. J. DIECKMANN, M.D., AND RUSSELL E. POTTINGER, S.M. (BY INVITATION), CHICAGO, ILL.

It has been noted by other investigators that when equilibrium is established after an injection of Sodium^{22} especially after some days, a certain amount of Sodium^{22} cannot be accounted for. It has also been reported that there is some parallelism between output and the excretion of Sodium^{22}.

Two normal subjects, one on a high sodium intake and the other on a relatively low sodium intake, collected 24-hour urines for a period of 58 days. The individual whose excretion of sodium ranged from 1 to 3 Gm. per 24 hours had a very gradual decrease in concentration of Sodium^{22} in the serum and a relatively slow excretion of Sodium^{22}. His sodium space was constant for about two weeks and at the end of 58 days only 6 per cent of his sodium could not be accounted for in the urine or serum. It has been shown that this amount is excreted in the feces.

The individual who was excreting from 4 to 8 Gm. of sodium per 24 hours had a more rapid decline in the concentration of Sodium^{22} in the serum, dropping to 9 per cent as compared with 28 per cent in Case 1 in 58 days. The sodium space began to increase markedly after the fourth day as compared with 22 days in Case 1. Twenty-four per cent of the Sodium^{22} was unaccounted for in Case 2 at the end of 58 days. After 3 months there was no detectable Sodium^{22} in Case 1 whereas in Case 2, 2.8 per cent could still be accounted for in the serum with only a trace in the urine. Similar observations are being made in pre-eclampsia.

43. HEPARIN EFFECT ON SERUM LIPOPROTEINS AS STUDIED BY FREE AND FILTER PAPER ELECTROPHORESIS

HAROLD L. DOBSON, M.D., AND SAMUEL H. STRIBLING, M.D., HOUSTON, TEX.

(INTRODUCED BY JAMES A. GREENE, M.D.)

Recently much attention has been focused on lipoproteins and their relationship to arteriosclerosis. One point of interest has been the dispersing effects of heparin on serum lipoproteins. As filter paper electrophoresis offers such advantages as simplicity and ability to study large numbers of patients, it was felt that it would be worth while to study the similarities and differences of free and filter paper electrophoresis before and two minutes after the intravenous injection of 50 mg. of heparin. Twenty individuals, both normal and abnormal, were studied. Free electrophoresis was studied with Perkin-Elmer apparatus, model 38, using schlieren diagrams. Paper electrophoresis was carried out by means of a modification of the Flynn and DeMayo procedure. Bromphenol blue and azocarmine were used to develop the total protein pattern while Sudan Black B and Sudan III techniques were used for lipoprotein studies. In general there was good agreement between the paper and free electrophoresis before the injection of heparin. There was some difference in the total protein patterns in the paper electrophoresis before and after heparin. However, there was a marked difference in the protein patterns of paper and free electrophoresis after the injection of heparin. In the free electrophoresis there was marked disruption of the pattern especially in the beta lipoprotein while with paper electrophoresis there was much less change.

Azocarmine tends to parallel the free electrophoresis more than does the bromphenol blue. When paper electrophoresis was stained for lipoprotein there were very few positive results with the technique used. These discrete positive areas in the beta globulin disappear after the injection of heparin.

It is felt that these results indicate some basic differences between free and filter paper electrophoresis which may prove useful in studying lipoproteins.

44. ABSORPTION OF Co^{60} LABELED VITAMIN B_{12} IN INTESTINAL BLIND LOOP MEGALOBLASTIC ANEMIA

ALFRED DOSCHERHOLMEN, M.D., AND PAUL S. HAGEN, M.D., MINNEAPOLIS, MINN.

(INTRODUCED BY EDMUND B. FLINK, M.D.)

A 32-year-old laborer was admitted to the hospital in August, 1952, with a history of progressive weakness and fatigue, intermittent glossitis, and numbness of the hands and feet of 6 months' duration. While in the Army he developed terminal ileitis, and an ileocolostomy was performed in April, 1944. Lack of improvement led to a second laparotomy in August, 1944, with closure of the loop of ileum distal to the anastomosis. Gradual recovery ensued, although he had 4 to 6 loose stools daily. On admission, physical examination was not remarkable. Blood examination revealed a macrocytic anemia with hemoglobin of 7.5 Gm. per 100 ml. of blood and a megaloblastic bone marrow. Gastric juice contained free hydrochloric acid. Fecal fat and nitrogen studies were normal. X-ray examination revealed an ileocolostomy with a blind loop of distal ileum and evidence of ileities. Vitamin C gave suboptimal response, while all blood values returned to normal on addition of vitamin B_{12} intramuscularly. He continued in remission with parenteral vitamin B_{12}. In March, 1954, the patient was restudied. Physical and laboratory findings were normal. X-ray examination again revealed the blind intestinal loop and some progression of the ileitis. B_{12}-Co^{60} absorption studies with 0.5 μg B_{12} orally resulted in fecal excretion of 78 per cent of the given dose and there was no urinary radioactivity after a "flushing" intramuscular dose of 1,000 μg of B_{12}.

The addition of 100 mg. of intrinsic factor concentrate failed to cause normal B_{12} absorption, although the same dosage had repeatedly proved effectual in pernicious anemia patients. Addition of 300 mg. of the intrinsic factor concentrate, pre-test saturation with oral B_{12} and attempted sterilization of the gut with neomycin failed to bring about normal absorption of the B_{12}-Co^{60} test doses. The patient's gastric juice effected some absorption of B_{12}-Co^{60} in a patient with pernicious anemia. Various possibilities to explain the failure of absorption of B_{12} in this patient will be discussed.

45. METABOLISM OF COMPOUND S IN RHEUMATOID ARTHRITIS: EFFECT ON 17-KETOSTEROID EXCRETION

IVAN F. DUFF, M.D., WILLIAM D. ROBINSON, M.D., WILLIAM M. MIKKELSEN, M.D. (BY INVITATION), AND ALETHA M. HEMERLINE, A.B. (BY INVITATION), ANN ARBOR, MICH.

Recent studies (J. LAB. & CLIN. MED. 43: 79, 1954) by Conn, Fajans, Louis, and Seltzer suggest that the excretory level of 17-ketosteroids can be modified by altered metabolism of adrenocortical steroids, and disclosed that patients

with liver disease excrete a much smaller proportion of administered compound S as 17-ketosteroids than do normal individuals. Oral administration of 400 mg. daily of compound S produces in normal subjects on the second day a peak increase in 17-ketosteroid excretion of 25 to 45 mg. above base-line values.

This test has been applied to 9 men and 7 women with rheumatoid arthritis. Base-line excretion of 17-ketosteroids was below normal in 12. Administration of compound S produced in only 5 patients a normal increment in 17-ketosteroid excretion (25 to 73 mg.); the response was subnormal in 3 (13 to 24 mg.). In the remaining 8, the rise in excretory level was 12 mg. or less. Further alteration in excretory pattern characterized by delay in peak values was seen in 8 patients. Liver function was normal in these patients with the exception of excessive Bromsulphalein retention in one. Initial response to compound S was not obviously correlated with duration, severity, or extent of the disease process.

The tests were repeated in 6 patients 8 to 12 months later. Clinical improvements in 3 subjects coincided with excretion of a larger proportion of administered compound S as 17-ketosteroids, in two of whom there had initially been no increase whatsoever. The clinical status of the remaining 3 was unchanged; the laboratory response was unchanged in 2 and had deteriorated in 1.

Similar altered metabolism of compound S has been observed in gout with Von Gierke's disease, rheumatoid spondylitis, pulmonary tuberculosis, in patients convalescing from poliomyelitis, and in some patients with gout.

46. THE SIGNIFICANCE OF THE PNEUMOTACHOGRAM IN THE STUDY OF THE MECHANICS OF VENTILATION

Richard V. Ebert, M.D., and Francis Haddy, M.D. (By Invitation),
Little Rock, Ark.

Various forces are involved in the movement of air in and out of the lungs. These forces include: (1) muscular action; (2) the elastic force of the lungs and chest wall; (3) the force required to accelerate the mass of the lungs, liver and chest wall; (4) the force required to overcome resistance to air flow in the air passages and frictional resistance to movement of the chest wall. It has been generally assumed that expiration during quiet breathing is passive and that the muscles are not involved. If this is true, study of the pneumotachogram during expiration should reveal much information regarding the mechanics of ventilation.

To elucidate this point, the pneumotachogram of anesthetized dogs given curare was studied. Curare was used to completely eliminate muscular action. The lungs were artificially inflated. When expiration was suddenly permitted, the rate of air flow rose almost immediately to a maximum value and then declined gradually. By integrating the pneumotachogram a volume curve was obtained. The difference between the total volume of air expired and the volume of air expired at time t was plotted. An exponential type of curve was obtained. These results imply that the pressure required to overcome acceleration of mass of the respiratory apparatus is negligible and that the pressure resulting from the elastic properties of the lung and chest wall and the pressure required to overcome the resistance to movement of air are approximately linear functions of change in volume and rate of air flow, respectively. The pneumotachogram of spontaneously breathing anesthetized dogs not given curare was markedly different from that of the animals paralyzed with curare.

The rate of air flow increased gradually to a maximum. Only the latter portion of the volume curve was exponential. The conclusion is that in the anesthetized animal only the latter portion of expiration is passive and unrelated to muscular action.

Study of pneumotachograms from unanesthetized human beings would indicate that all of expiration is influenced by muscular action. As a result, analysis of the pneumotachogram obtained during quiet breathing is of no aid in the study of the mechanics of ventilation.

47. URINARY CALCIUM EXCRETION AS A MEANS OF MEASURING EFFECTIVENESS OF ENDOCRINE MANAGEMENT OF METASTATIC CARCINOMA OF THE BREAST

NYLENE ECKLES, M.D. (BY INVITATION), AND SAMUEL G. TAYLOR, III, M.D., CHICAGO, ILL.

About seventy-five ambulatory patients with metastatic cancer of the breast were observed during steroid hormone deprivation and administration. The urinary calcium excretion was determined at various periods before and after the administration of testosterone, estrogens or cortisone in some, and before and after castration or adrenalectomy in others. This has been correlated with clinical response, tumor growth, serum alkaline phosphatase, and roentgen changes.

48. HEPATIC CIRCULATION AND FUNCTION IN PATIENTS WITH MITRAL STENOSIS

JOHN W. ECKSTEIN, M.D. (BY INVITATION), JAMES W. CULBERTSON, M.D., AND GEORGE N. BEDELL, M.D. (BY INVITATION), IOWA CITY, IOWA

In patients with congestive heart failure a decrease in hepatic blood flow (EHBF) parallels the reduction in cardiac output, and hepatic extraction (E_{bsp}) and plasma clearance (C_{bsp}) of Bromsulphalein are diminished (Myers). Patients with mitral stenosis who are not in heart failure tend to have a low cardiac output. This study was undertaken to determine whether hepatic circulation and hepatocellular function are normal in such cases.

Observations were made on ten patients with mitral stenosis. Seven of these (2 men, 5 women) had no systemic venous congestion (normal hepatic venous, right atrial and right ventricular end-diastolic pressure levels). Cardiac index (Fick) was low (2.3 to 2.5) in three patients, low normal (2.7 to 3.1) in three, and normal (3.6) in one (mean, 2.8 L./min./M^2). Two patients had low normal (1,193, 1,352) EHBF, and five had normal (1,396 to 1,710) values (mean, 1,487 ml./min./1.73 M^2). C_{bsp} was low (275, 307) in two subjects, normal (404 to 730) in five (mean, 461 ml./min./1.73 M^2). E_{bsp} was low (0.29 to 0.33) in the two patients with low C_{bsp}, and normal (0.41-0.82) in five (mean, 0.54). Splanchnic oxygen consumption (SpO_2) was low (47) in one patient, normal (66 to 89) in five, and increased (122) in one (mean, 77 ml./min./1.73 M^2).

Two men with mitral stenosis, not in heart failure (normal right ventricular end-diastolic pressure) but with tricuspid stenosis (elevated right atrial and hepatic vein pressure), had normal (3.2, 3.4) cardiac indices and normal (1,420, 1,482) EHBF. C_{bsp} was normal (447) in one and subnormal (340) in one. E_{bsp} was normal (0.54) in the first and low (0.33) in the latter. SpO_2 was normal (68) in the first (not determined in the latter).

One man with mitral stenosis and frank right ventricular failure had the expected uniformly low values throughout.

These observations demonstrate normal hepatic circulation and function in mitral stenosis patients not in right ventricular failure. They show that impaired liver function may accompany mild tricuspid stenosis with central systemic venous congestion. They confirm reduction of both hepatic circulation and hepatocellular function in frank right ventricular failure.

49. METABOLISM OF CORTISONE BY RAT LIVER HOMOGENATE

ALBERT B. EISENSTEIN, M.D., ST. LOUIS, MO.

(INTRODUCED BY ROBERT E. SHANK, M.D.)

The liver is the major site of metabolism of adrenocortical steroids including cortisone. Reduction of the conjugated, unsaturated bonds in ring A of the molecule and degradation of the 17,21-dihydroxy-20-ketone side chain are among the changes that occur during the hepatic metabolism of cortisone. Although these changes are enzymatic, little is known of the enzyme systems concerned. The present study was carried out in an attempt to determine some of the characteristics of these enzymes.

Rat liver homogenates were incubated in a physiologic, buffered medium containing cortisone as substrate. After incubation the steroids were recovered and analyzed for the presence of conjugated, unsaturated bonds in ring A by ultraviolet spectroscopy and the sulfuric acid technique of Zaffaroni. The presence of a dihydroxy-ketone side chain was determined by the Porter-Silber method.

Incubation of liver homogenate with cortisone for one hour at 37° C. resulted in an average loss of 20 per cent of the conjugated, unsaturated bonds and average degradation of 26 per cent of the dihydroxy-ketone side chain. Addition of citrate or iso-citrate to the incubation mixture in 0.01 M concentration resulted in an increased rate of disappearance of both the conjugated, unsaturated system and the side chain. Diphosphopyridine nucleotide (DPN) accelerated destruction of the conjugated double bonds but only slightly increased the degradation of the side chain. Following addition of 0.01 M alpha ketoglutarate there was inhibition of reduction of the alpha-beta unsaturated ketone and degradation of the dihydroxy-ketone structure. Other compounds including succinate, pyruvate, ATP, AMP, and versene had no influence on the rate of either reaction.

Since versene, a metal-binding agent, and succinate, an intermediate in Kreb's cycle, had no influence on the metabolism of cortisone, it is believed that citrate is a required cofactor in the metabolism of this steroid. The mechanism by which DPN accelerates reduction of the conjugated, unsaturated bonds is not apparent. The inhibition of steroid metabolism following addition of alpha ketoglutarate may result from competition between it and the steroid for the DPN in the system. The possible clinical significance of these observations will be discussed.

50. THE EFFECT OF STRESS AND CORTISONE ON THE CIRCULATING THYROID HORMONE

WILLIAM W. ENGSTROM, M.D., AND BLANCH MARKARDT, M.A. (BY INVITATION), MILWAUKEE, WIS.

It has been shown that environmental stress or the administration of cortisone will depress the radioiodine uptake by the thyroid of animals. Also.

Bondy and Hagewood (Proc. Soc. Exper. Biol. & Med. **81**: 328, 1952) suggested that cortisone decreases the rate of peripheral utilization of thyroid hormone.

In preliminary studies we have demonstrated in 24 patients that uncomplicated surgical stress of varying severity does not alter the concentration of the circulating thyroid hormone as measured by the serum precipitable iodine (SPI). Thus, the SPI should be a reliable measure of thyroidal activity even in the acutely ill patient.

To eliminate endogenous variations in thyroid function, cortisone in large amounts was acutely administered for two or three days to patients with primary myxedema or anterior pituitary insufficiency while they were maintained on exogenous desiccated thyroid. No change in SPI occurred. Under these circumstances in man there was no evidence of an altered rate of disposal of thyroid hormone by cortisone.

However, while acute uncomplicated stress (surgery) or the acute administration of cortisone does not alter the SPI, patients suffering from serious, chronic illnesses frequently exhibit abnormally low SPI. Values as low as those seen in severe myxedema are not infrequently encountered. In these the SPI is correlated with the level of the serum albumin. Conceivably, the SPI could be depressed if the thyroxine, normally bound firmly to protein, were to exist in a free state or in an easily dissoluble form. However, we found the butanol-extractable iodine also to be low in these instances. Thus, malnutrition and chronic illness in some way may lead to a true depression of the circulating thyroid hormone.

51. ADRENAL HORMONE THERAPY IN VIRAL HEPATITIS. IV. THE EFFECT OF GAMMA GLOBULIN AND ORAL CORTISONE IN THE ACUTE DISEASE

ALFRED S. EVANS, M.D., COL. ROBERT S. NELSON, MC (BY INVITATION), LT. COL. HELMUTH SPRINZ, MC (BY INVITATION), AND CAPTAIN FRANK P. CANTRELL, MC (BY INVITATION), MADISON, WIS.

Earlier studies have established that both ACTH and cortisone significantly influence the course of acute viral hepatitis compared to patients treated only with rest and a nutritious diet. A prompt fall in bilirubin and a definite improvement in appetite were noted. With cortisone in particular, the primary recovery period was shortened by two weeks and histopathologic studies of liver biopsies revealed more rapid progress toward healing. The principal disadvantage to therapy was the striking incidence of relapses of the order of 20 per cent as compared to none in a control group. In addition, such treatment was both prolonged (28 days) and painful because of the intramuscular route.

The main purpose of the present study was to determine whether the administration of gamma globulin in conjunction with cortisone could lower the relapse rate. This was based on the premise that gamma globulin might bolster immunity in a patient made more vulnerable to relapses by hormone therapy. Secondarily, we wished to learn if this compound was effective if given orally and if a shorter treatment schedule could be employed.

Twenty-two patients with acute viral hepatitis were given oral cortisone in conjunction with the administration of large amounts of gamma globulin intramuscularly at the beginning and end of hormonal therapy. Gamma globulin failed to prevent relapses: four occurred, an incidence similar to that in ACTH or cortisone treated subjects without conjunct gamma globulin therapy.

Oral cortisone produced a rapid initial drop in bilirubinemia but failed to influence significantly the total duration of illness except in two patients receiving therapy over a three-week period.

Even under ideal circumstances cortisone does not shorten the duration of disease sufficiently, in our opinion, to run the increased risk of relapse, and thus is not recommended for the routine treatment of viral hepatitis. It may have a place in the treatment of severe, markedly symptomatic, and progressive viral hepatitis and certainly deserves further trial in fulminant hepatitis with coma.

52. ELECTROLYTE CHANGES IN DIFFERENT PARTS OF THE GASTROINTESTINAL TRACT DURING RESTRICTION OF DIETARY SODIUM WITH AND WITHOUT THE ADMINISTRATION OF CATION-EXCHANGE RESIN

H. Field, Jr., M.D., Leon Swell, Ph.D. (By Invitation), R. E. Dailey, M.S. (By Invitation), E. C. Trout, Jr., B.S. (By Invitation), and R. S. Boyd, M.D. (By Invitation), Martinsburg, West Va.

The suggested failure of cation-exchange resins to combine with "endogenous" sodium does not explain the inefficacy of such resins in patients whose dietary sodium is much restricted. We have contrasted the average fecal sodium of 0.48 meq. and fecal potassium of 1.52 meq. per gram of resin, in long-term observations on patients with dietary sodium moderately restricted to 35 to 52 meq. per day, while taking 15 Gm. of a carboxylic exchange resin with each meal, with the uptake of 2.34 meq. of sodium and 0.18 meq. of potassium, per gram of resin, when in equilibrium, in vitro, with the average electrolyte composition of the terminal ileum. Those differences could be due to changes in the electrolyte composition in the terminal ileum, accompanying the restriction of dietary sodium, or to dynamic absorption of sodium, with lack of such absorption of potassium, by the colon.

Experiments, utilizing dogs with fistulas into their terminal ileum or into their jejunum, demonstrate that both those possibilities occur in the dog.

During the simple, dietary restriction of sodium, diet otherwise identical, the sodium concentration in the terminal ileum declined from about 100 meq. per liter to about 10 meq. per liter. That indicates an efficient conservation of sodium in the upper gastrointestinal tract. There was a corresponding, reciprocal increase in the potassium concentration in the terminal ileum. That suggests a dynamic mechanism for selective absorption, or secretion, or both, of those ions, which tends to maintain a constant osmolarity of the ileal contents.

When 15 Gm. of resin were administered after each day's feeding, a diet with "normal" sodium content, and at three different levels of dietary sodium restriction, diet otherwise identical, following control periods, before resin administration, at each level of dietary sodium, there was a step-wise decline in the amount of sodium bound per gram of resin, with each decrease in the dietary sodium.

At each level of dietary sodium, there was $\frac{1}{3}$ to $\frac{1}{16}$ as much sodium bound in the feces, per gram of resin, as was bound by the resin in the terminal ileum. That indicates dynamic absorption of sodium by the colon.

53. ENERGY COST OF THE MASTER TWO-STEP TEST

AMASA B. FORD, M.D. (BY INVITATION), WILLIAM K. LITTMAN (BY INVITATION), HERMAN K. HELLERSTEIN, M.D., AND ARISTIDES GOUSIOS (BY INVITATION), CLEVELAND, OHIO

The electrocardiographic changes of Master's two-step test are familiar, but the energy cost of the test has not been previously determined. In the present study this cost was measured in 52 healthy male subjects between the ages of 20 and 69 years. Oxygen consumption was determined by the open circuit method, and electrocardiograms were taken during resting, exercise, and recovery periods.

In the normal subjects mean total oxygen consumption in excess of resting was 2.32 ± 0.34 liters (1.50 liters per minute of exercise). Mean work performed was 765.9 ± 78 kilogrammeters (494 kilogrammeters per minute of exercise). The ratio of oxygen consumption to work performed was greater ($p = < 0.01$) in the subjects over age 40 than in those below 40, but the total oxygen consumption was not significantly different. "Respiratory efficiency" (cubic centimeters of oxygen extracted per 100 ml. of air breathed) was greater ($p = < 0.01$) at rest in the younger group, but not during exercise. In all cases the pulse rate returned to within ten beats of the resting level by two minutes after exercise, and 86 ± 9 per cent of the total oxygen debt had been repaid in three and a half minutes. Electrocardiograms before and after exercise were normal.

The two-step test was compared with an identical total ascent on a standard stairway in the same period of time (1½ minutes). Stair-climbing required 12 per cent less oxygen consumption than the step test. The difference is significant ($p = < 0.01$) and is attributed to more frequent turning involved in the step test.

Preliminary studies have been made on 18 patients, 8 with hypertension, and 10 with arteriosclerotic heart disease and myocardial infarction. "Respiratory efficiency" during exercise was lower than normal, and in 13 of 18 cases blood pressure and pulse rate failed to return to resting levels in two minutes. The electrocardiogram changed after exercise in only 3 of 18 cases.

Summary.—The two-step test requires a large energy expenditure which is equal in all age groups. Changes in blood pressure, pulse rate, and "respiratory efficiency" appear to be more sensitive parameters of cardiovascular function than are electrocardiographic changes.

54. CYCLIC VARIATIONS IN OXYGEN SATURATION OF ARTERIAL AND CENTRAL VENOUS BLOOD IN PATIENTS WITH INTRACARDIAC OR GREAT VESSEL SHUNTS

IRWIN J. FOX, M.D., (BY INVITATION), HOWARD B. BURCHELL, M.D., AND EARL H. WOOD, M.D., PH.D., ROCHESTER, MINN.

Variations in oxygen saturation of blood with respiratory or cardiac cycles in patients with shunts are of interest since they might be used to study changes in the magnitude and direction of shunts produced by breathing or the heartbeat. If present, these variations could produce significant errors in measurement of systemic or pulmonary blood flow by conventional applications of the Fick principle.

In normal subjects and cardiac patients fluctuations in arterial oxygen saturation were studied by means of continuously recording earpiece oximeters and a cuvette oximeter attached to a needle in the radial artery. Variations in oxygen saturation of central venous blood were investigated by constant withdrawal of blood at a rapid rate through a recording cuvette oximeter via a cardiac catheter. Particularly for the study of variations at cardiac frequency, an electrical synchronizer assembly was used permitting withdrawal of blood during any desired portion of the cardiac cycle.

Variations in arterial oxygen saturation at respiratory frequency were usually not detected in normal subjects but characteristic changes were produced by breath holding in the inspiratory and expiratory positions. Variations, however, were frequently found in patients with right-to-left shunts who also may show paradoxical effects with voluntary alteration in breathing.

Variations in oxygen saturation of central venous blood at respiratory frequency were detected in all normal subjects studied. These could be abolished by breath holding and were exaggerated by deep breaths and usually by exercise. They were uniformly greatest (0.5 to 5 per cent) in the inferior cava above the renal veins.

Similar variations were usually detected in patients with shunts. In some with bidirectional shunts the largest fluctuations were found at the shunt. A striking parallelism in fluctuations in oxygen saturation of arterial and venous blood beyond the shunt indicated that left-to-right and right-to-left components were affected simultaneously in inverse directions.

The dynamic response characteristics of the catheter-cuvette-oximeter blood sampling assembly prevent estimation of the true maximal amplitude of the cyclic variations even at respiratory frequency. Hence significant errors in determinations of systemic and pulmonary flow by conventional Fick methods are possible.

55. ADRENOCORTICOTROPIN AND CORTISONE IN THE TREATMENT OF SEVERE REITER'S SYNDROME

Donald T. Foxworthy, M.D., Robert M. Poske, M.D. (By Invitation), Evan M. Barton, M.D., and Max M. Montgomery, M.D., Chicago, Ill.

(Introduced by Louis R. Limarzi, M.D.)

Reiter's syndrome, a condition of undetermined etiology, is manifested by urethritis, conjunctivitis, and arthritis, and in its moderate and severe forms follows a characteristic course over a two to four month period. Remissions occur within a few weeks in the milder type of this syndrome. The patients with the severe form have persistent joint and muscle pain, high fever, rapid weight loss, marked joint effusions, and some also have extensive mucous membrane and skin lesions. These patients sometimes present a difficult problem in management.

Seven such patients have been treated with ACTH and/or cortisone, plus the usual conservative measures. Total treatment per patient ranged from 14 days to 93 days. Response was prompt and symptoms were usually well controlled on a moderate dosage schedule. One patient required large amounts of ACTH. Another patient on ACTH developed marked apprehension so bothersome that he preferred the joint symptoms to continuance of therapy. The dosage in all patients was kept at the lowest level which would control

severe manifestations of the disease. Reduction was carried out slowly, and treatment was discontinued when the dosage reached levels ordinarily considered to be ineffective.

The duration of the syndrome was not shortened by the use of these substances, but convalescence appeared to be more rapid since there was less weight loss and debility in the treated patients. There were no complicating intercurrent infections, and the basic inflammatory process did not appear to be adversely influenced.

It is our opinion that hormone therapy, while not curative, represents the best available form of symptomatic treatment in severe Reiter's syndrome.

56. EVALUATION OF NASAL EOSINOPHILS IN PEDIATRIC HEALTH APPRAISALS

GEORGE S. FRAUENBERGER, M.D., EVANSTON, ILL.

(INTRODUCED BY THERON G. RANDOLPH, M.D.)

Eosinophil nasal smear determinations were performed routinely in a pediatric practice during a two-year period for the purpose of objectively establishing the existence of clinical allergic manifestations. These observations were made during the course of routine health appraisals. Findings were correlated with routine health appraisals, in which growth was evaluated by comparing the rate of growth of five standard measurements in terms of their percentile deviations. Additional nasal smear determinations were made intermittently on parents.

Nasal mucus for the preparation of thick smears was obtained by means of inserting a tissue taper in the floor of each nasal cavity. Hansel's Giemsa technique was employed, using a freshly prepared dilution of the stain each day.

As the seasonal variation in the percentage of eosinophils in the nasal smears of both children and parents were similar, these data will be presented in totals for the entire group. The percentage of positive smears was lowest in July (39.2) and highest in January (73.3), with graded variations existing between these two extremes. Of the total of 2,269 determinations, 59.5 per cent were positive for eosinophils.

In those instances in which nasal smears were consistently negative, growth proceeded normally with relatively few interruptions from infectious processes or other illnesses. Those children with the greatest percentage of nasal eosinophils exhibited the poorest growth. There was no consistent relationship between the nasal eosinophils of the children and those of parents with the exception that high eosinophil values in parents correlated with highly positive nasal eosinophil findings in their offspring. One unanticipated aspect of this study was the fact that a high percentage of eosinophils in newborn infants served as an aid in indueing mothers to breast feed their babies. In general, eosinophils in the nose disappear during infections, returning as an indication of recovery; this emphasizes the desirability of making repeated nasal smears per patient rather than depending upon a single observation.

Sufficient information accrued from this study to warrant the continuation of periodic nasal eosinophil determinations as a part of routine pediatric health appraisals.

57. PROTEIN DIGESTION STUDIES IN PATIENTS WITH PANCREATIC DISEASE USING I^{131}-LABELED HUMAN SERUM ALBUMIN

ROBERT J. FREEARK, M.D. (BY INVITATION), AND DONALD D. KOZOLL, M.S., M.D., CHICAGO, ILL.

Previous studies have shown that impaired protein digestion in persons with marked pancreatic insufficiency may be determined by measuring blood levels of isotope following a test meal utilizing iodinated^{131}-labeled protein (IHSA). The present study is concerned with the practical application of this test in the study of patients with various forms of pancreatic disease as encountered in a large general hospital.

The methods of the test have been previously described. A meal, consisting of gelatin, iodinated ^{131} human serum albumin and distilled water, is given to patients fasted for twelve hours. Venous blood samples were obtained at hourly intervals and assayed by means of a well type scintillation counter for radioactivity. The results are expressed as percentage of ingested isotope present in estimated total blood volume. Although peak levels of concentration were usually obtained at two hours, blood specimens were collected beyond this point to ensure correct appraisal of protein absorption.

Among the pancreatic diseases studied were acute, subacute and chronic relapsing pancreatitis (20 cases), carcinoma of head of the pancreas (6 cases), carcinoma of the body and tail of the pancreas (4 cases), carcinoma of the ampulla of Vater (3 cases), pancreatolithiasis (3 cases), and subtotal pancreatic resection (1 case). In addition, nonpancreatic obstructive jaundice (8 cases) and chronic diarrheas other than pancreatic in origin (6 cases) were included.

The use of an I^{131}-labeled protein digestion study in patients suspected of pancreatic disease has limited but definite practical value. Localized disease of the pancreas without proximal pancreatic duct obstruction gave blood isotope concentrations similar to controls. In patients with chronic diarrhea pancreatic insufficiency can be ruled out on the basis of this test but the isotope curves are not diagnostic. Pancreatitis in the acute, subacute, and chronic forms, when the latter is not associated with diarrhea, is not characterized by abnormal protein digestion as measured by this test. In cases of obstructive jaundice associated with pancreatic duct obstruction, the preoperative localization of the offending mechanism in the head of the pancreas is possible by means of this test.

58. ACQUIRED CIRCULATING ANTICOAGULANTS IN SYSTEMIC "COLLAGEN DISEASE"

REPORT OF THREE CASES WITH ONE INSTANCE OF TRANSPLACENTAL TRANSFER OF THE ANTICOAGULANT

PAUL G. FRICK, M.D., MINNEAPOLIS, MINN.

(INTRODUCED BY C. J. WATSON, M.D.)

Two patients with disseminated lupus erythematosus and one patient with systemic manifestations secondary to a drug reaction were found to have a coagulation defect caused by a circulating anticoagulant. Studies of hemo-

stasis revealed a prolongation of the clotting time, prothrombin time, and recalcification time. The consumption of prothrombin was markedly impaired. The bleeding time was prolonged in only one case. Platelets, fibrinogen concentration, and clot retraction were normal. Mild hypoprothrombinemia was observed in all cases when tested with the two-stage method. It was demonstrated that the anticoagulant probably inhibited thromboplastin. The inhibitor was not heparin. It resisted heating at 60° C. for 30 minutes, was not dialyzable, and could not be absorbed on $BaSO_4$.

In all three cases the anticoagulant was associated with a false positive blood Wassermann reaction and a 4+ cephalin cholesterol flocculation. The newborn baby of one patient with lupus erythematosus disseminatus was found to have the anticoagulant for 7 weeks; the baby also had a false positive serology and an abnormal cephalin cholesterol flocculation for 6 months after birth. The transplacental transfer of the anticoagulant and its association with other manifestations of antigen-antibody reactions strongly suggests that the mechanism of its development is immunologic in type.

59. 2-HYDROXYSTILBAMIDINE IN THE THERAPY OF SPOROTRICHOSIS

JOSEPH E. GERACI, M.D. (BY INVITATION), JOHN A. ULRICH, PH.D. (BY INVITATION), THOMAS J. DRY, M.B., LYLE A. WEED, M.D. (BY INVITATION), GEORGE P. SAYRE, M.D. (BY INVITATION), AND COLLIN S. MACCARTY, M.D. (BY INVITATION), ROCHESTER, MINN.

A 60-year-old man was seen with the rare syndrome of generalized (visceral) sporotrichosis including involvement of the cerebrum and spinal cord. For 17 years the patient had been employed in a cement, tile, and clay products company, a job associated with much dust and contact with many imported clays. Diagnosis was established by culture of *Sporotrichum schenckii* from one of the many subcutaneous nodules. The patient was treated with 200 to 250 mg. of 2-hydroxystilbamidine daily for a period of 32 days. The development of allergic sensitivity to the medication precluded its further use. Attempts to readminister the drug in solution with an antihistaminic resulted in an anaphylactic shocklike picture. With 2-hydroxystilbamidine therapy both subjective and objective improvement occurred and persisted for some 2 weeks after the use of the drug was discontinued. When 2-hydroxystilbamidine could not be administered any longer, the patient was treated with iodides, the daily dose being increased to tolerance. The patient, however, deteriorated and died after 107 days of observation and treatment at the clinic and after approximately 150 days of active disease. Extensive post-mortem studies were carried out and will be reported.

In vitro and in vivo studies with the strain of *Sporotrichum schenckii* isolated from the patient were carried out. Suspensions of the organism were made and injected into mice intraperitoneally. Treatment with 2-hydroxystilbamidine was started 48 hours after inoculation. One milligram of the drug per day was injected intraperitoneally for a period of 14 days. The mice were examined by slide preparations and by culture. The proper organism and drug controls in mice were carried out.

60. THE HEMODYNAMICS OF SHOCK DUE TO INFECTION

ROBERT P. GILBERT, M.D., KLAAS P. HONIG, M.D., BERNARD H. ADELSON, M.D., JOSEPH A. GRIFFIN, M.D., AND ROBERT BECKER, M.D., CHICAGO, ILL.

(INTRODUCED BY CRAIG W. BORDEN, M.D.)

Shock due to infection is of clinical importance since it may become resistant to treatment. It has also been considered as a reason for hemorrhagic shock to become irreversible. The blood volume has been shown to be normal in this condition, but few determinations of the cardiac output have been reported.

Five patients with shock due to infection were studied. Bacteremia was proved in four cases: Pneumococci were isolated in two, meningococci in the third and *E. coli* in the fourth. The fifth case was one of severe pneumonia from which a blood culture was not drawn. The dye-dilution technique for cardiac output was used in four cases and the direct Fick principle in the fifth. The arterial pressure was recorded directly from the femoral artery. The average arterial pressure was 82/53 mm. Hg with an average mean pressure of 63 mm. Hg. In four cases the cardiac index averaged 3.6 L./min. M^2 (Range 3.0 to 4.2 L./min. M^2). Although height and weight figures were not obtained in the fifth patient, the cardiac output was 3.9 L./min. so the cardiac index was presumably below normal. The average total peripheral resistance was accordingly reduced. The dye circulation times were normal or slightly prolonged in the four cases measured.

The action of norepinephrine was investigated in four cases. The arterial pressure and total peripheral resistance increased in all cases and the cardiac output fell slightly in three instances. Concentrated serum albumin (12.5 Gm.) was infused in the fifth case of this series and was followed by an increase of cardiac output from 3.9 to 4.6 L./min. and a 320 ml. rise of plasma volume, but no change in hematocrit.

It is apparent that the hypotension of septic shock may be associated with a cardiac index within the usual normal limits. The total peripheral resistance was reduced, presumably as a result of vasodilatation. Norepinephrine infusion was followed by an arterial pressure rise but no increase of cardiac output.

61. ASSAY OF PLASMA PROTHROMBIN WITH A SYNTHETIC SUBSTRATE: ITS COMPARISON WITH STANDARD METHODS OF PROTHROMBIN ASSAY

HELEN I. GLUECK, M.D., AND SOL SHERRY, M.D., CINCINNATI, OHIO

It has been demonstrated that thrombin enzymatically hydrolyzes arginine esters. A substituted arginine ester, toluenesulfonyl-l-arginine methyl ester (TAMe) has been found particularly suitable for the measurement of thrombin, and has been utilized in the development of a quantitative assay for plasma prothrombin. The TAMe assay measures the actual prothrombin in the plasma, since it measures prothrombin in terms of yield, rather than time. One TAMe unit approximates one National Institute of Health clotting unit.

Observations on a series of adults reveal that normal human plasma contains 53 ± 4 TAMe units of prothrombin per milliliter, with little daily variation. No instance of hyperprothrombinemia has been observed in patients with thromboembolic disease. In animals exhibiting rapid prothrombin times, the prothrombin concentration as measured by TAMe is comparable to that

observed in human beings, suggesting that rapid prothrombin time in animals is due to accelerator factors rather than an elevated prothrombin concentration.

The TAMe assay shows that in Ac-globulin (labile factor) deficient human plasma, the rate of activation of prothrombin is delayed, but the final yield of thrombin is eventually complete, whereas with conventional techniques a normal thrombin yield is not obtained.

When the plasma prothrombin time of dicumarolized patients and animals is measured simultaneously by the TAMe, one- and two-stage assays, close correlation is observed between the TAMe and two-stage methods. By contrast, the prothrombin time as measured by the one-stage assay, falls more rapidly with dicumarol therapy and rises more rapidly on discontinuing the drug. When dicumarolized animals and patients are given vitamin K_1 (Mephyton) the one-stage method shows a more rapid rise in prothrombin time than does the TAMe assay. These observations suggest that in addition to their effect on prothrombin, dicumarol and vitamin K_1 affect a plasma factor which accelerates the one-stage assay. This accelerator is more sensitive to the effect of dicumarol and vitamin K_1 than prothrombin itself. The addition of Ac-globulin to dicumarolized plasmas, however, does not alter the differences between the TAMe and one-stage assays.

These experiments exemplify the utility of a synthetic substrate for prothrombin assay.

62. GLUTAMIC ACID IN THE TREATMENT OF HEPATIC COMA

ROBERT GOLDBLOOM, M.D. (BY INVITATION), SAMUEL HYMAN, M.D. (BY INVITATION), AND FREDERICK STEIGMANN, M.D., CHICAGO, ILL.

Recent reports in the British literature on the successful treatment of hepatic coma with glutamic acid raised the hope that at last an agent had been found which might alter the fatal course of this condition. We, therefore, decided to test the value of this amino acid in the treatment of hepatic coma in some of these cases admitted to the Cook County Hospital.

Ten patients who had been in hepatic coma resulting from cirrhosis of the liver for from six hours to several days were given daily a 20 per cent solution of sodium glutamate in a buffered diluent. One hundred milliliters of the sodium glutamate was added to 1,000 ml. of 5 per cent glucose in distilled water, and this mixture was given by slow intravenous infusion. Some patients who were critically ill were given 3 liters of this mixture in a twenty-four-hour period.

Our results with glutamic acid in the treatment of hepatic coma were extremely poor. Two patients who were in coma cleared slightly and appeared improved initially, only to die two days later, following massive hematemeses. Two patients treated with glutamic acid remain well at the time of this report. One of these patients had been in coma six hours prior to the use of glutamic acid while the other had been comatose for about twenty-four hours. The remaining six patients died while under active treatment with glutamic acid without regaining consciousness. Any improvement noted appeared within six to twelve hours after start of the treatment in contrast to the patients reported in the British literature, who showed dramatic improvement three to four hours after starting the infusion of glutamic acid.

These results seem to indicate that glutamic acid, which plays a role in the metabolism of ammonia, raised the level of consciousness in patients with liver disease who were lethargic or on the verge of going into hepatic coma, but was of little benefit in our patients, comatose for several days.

63. CLINICAL INVESTIGATIONS IN PATIENTS WITH AGAMMAGLOBULINEMIA

ROBERT A. GOOD, PH.D., M.D., MINNEAPOLIS, MINN.

During the past year we have studied seven patients with agammaglobulinemia. Two were adults having the acquired disease and five were children suffering from the congenital form. Our observations support the contention that congenital agammaglobulinemia is an inborn error of metabolism transmitted as a sex-linked recessive trait. The clinical syndrome observed was featured by extreme susceptibility to infection, each of the patients studied having had many severe bacterial infections.

Recognition and demonstration that patients with agammaglobulinemia have a complete or virtually complete "paralysis" of the immunologic mechanism permits incisive investigation of many immunologic problems. Our inquiry into a number of the latter provides the basis of this report.

Comparison of hematopoietic tissues in patients with agammaglobulinemia with those of normal children and adults revealed, in agammaglobulinemia, a relative deficiency of lymphoid cells and a virtual absence of plasma cells. Employing serial aspiration biopsies of the bone marrow and surgical biopsy of draining regional lymph nodes the morphologic response to antigenic stimulation of normal children, normal adults, and patients with agammaglobulinemia was compared. Whereas the normal subjects all reacted with plasmacytosis, more striking during the secondary response, patients with agammaglobulinemia produced neither plasma cells nor antibody in response to initial and secondary contact with antigen.

Additional studies have established the following points: (a) Skin and systemic reaction regularly occur in agammaglobulinemic patients following injection of gram-negative bacterial endotoxins. (b) Refractoriness to effects of gram-negative bacterial endotoxin develops in patients with agammaglobulinemia in spite of their inability to produce demonstrable circulating antibody. (c) Acute-phase proteins (c-reactive protein, mucoprotein) are produced in patients with agammaglobulinemia in response to appropriate stimulation, an observation which tends to dissociate these events from antibody production in man. (d) Homotransplantation of skin has been successful in a child with agammaglobulinemia. (e) Bacterial type hypersensitivity against streptokinase-streptodornase is lacking in patients with agammaglobulinemia and present in 80 per cent of normal persons. Transmission of bacterial type hypersensitivity to streptokinase-streptodornase but failure of agglutinin formation in agammaglobulinemia upon injection of viable white blood cells from persons hypersensitive to streptokinase-streptodornase who were forming antibody against typhoid organisms has been observed.

64. INACTIVATION OF SALT-RETAINING HORMONE BY LIVER

EDGAR S. GORDON, M.D., MADISON, WIS.

The liver is known to metabolize many steroid compounds, including estrogens, androgens and adrenal cortical hormones with resulting alteration in their biological potencies. The association of demonstrable liver functional impairment in human subjects with ascites and edema who excrete pathologically large amounts of salt-retaining hormone in the urine suggested, as a possible underlying mechanism, a failure of normal liver inactivation of mineralocorticoid compounds of adrenal origin. This probability is substantiated by our inability to produce these high titers in urine in normal subjects through intense adrenal stimulation with ACTH.

Surviving rat liver slices have been incubated in Warburg flasks, both aerobically and anaerobically, with active sodium-retaining extracts obtained from twenty-four-hour urine collections from patients with cirrhosis of the liver and ascites. After 90 to 120 minutes, contents of the flasks were re-extracted and reassayed for sodium-retaining activity.

Actively respiring liver slices consistently destroy 90 to 100 per cent of sodium-retaining activity and anaerobic conditions significantly impair this capacity. Boiled liver slices produce no inactivation. Kidney slices, though actively respiring, have very little effect. Slices of normal human liver obtained at biopsy behave exactly like the rat tissues.

Tentative evidence is thus provided which suggests that sodium retention in several pathologic conditions results from a failure of a specific liver function. Accumulation of sodium-retaining hormone so produced is believed to be responsible, in part at least, for the accompanying fluid-retention characteristic of these diseases. The steroid compound involved is thought to be aldosterone or a derivative of it.

65. THE PATHOGENESIS AND PATHOLOGY OF EXPERIMENTAL HISTOPLASMOSIS INFECTIONS IN MICE

J. Thomas Grayston, M.D., and Philip Altman, M.S., Chicago, Ill.

(Introduced by Clayton G. Loosli, M.D.)

In this study 3- to 4-week- and 8- to 16-week-old CFW strain of white mice were employed. Routes of inoculation were intranasal (under light anesthesia), intraperitoneal, and oral (in drinking water). The inoculum consisted of suspensions of mycelial phase histoplasma with a measured number of viable spores determined by comparison of hemocytometer spore counts and the number of colonies growing on blood agar plates. Mice were sacrificed at intervals after inoculation for cultural and pathologic studies of the lung, liver, and spleen. Mortality data were obtained from a separate series of experiments in which spontaneous deaths were recorded over thirteen to fifteen months of observation.

Mice proved to be extremely resistant to the oral route of inoculation as no infections were demonstrated by culture after ingestion of 100,000 spores. Forty per cent of mice given 10,000,000 spores orally were culturally positive. When mice were inoculated with 100 or more spores intranasally or intraperitoneally over 90 per cent became infected, while after 10 spores approximately 50 per cent became infected. Infected mice surviving up to fifteen months still showed positive cultures.

There was somewhat greater survival in intranasally infected mice given ten spores (19 per cent) compared with 1,000 and 100,000 spore groups (9 and 6 per cent, respectively). However, in all three groups the mortality pattern was similar with 40 to 50 per cent of the deaths occurring during the fourth and fifth months after inoculation and another 30 to 35 per cent being observed in the combined third, sixth, seventh, and eighth months. Similar patterns were found for both young and old mice and after intraperitoneal inoculation.

Lung lesions observed one week after intranasal inoculation failed to progress, but showed granulomatous organization during the first month and resolution and disappearance by two to four months. In the liver and spleen following intranasal or intraperitoneal infection small collections of round cells were observed by two weeks. These granuloma continued to enlarge

and coalesce. Multinucleated giant cells loaded with *H. capsulatum* appeared between one and two months. By four to eight months the lesions frequently replaced most of the liver parenchyma.

66. CLINICAL EVALUATION OF ANALGESICS

CHARLES M. GRUBER, JR., M.D., INDIANAPOLIS, IND.

(INTRODUCED BY K. G. KOHLSTAEDT, M.D.)

In five midwestern hospitals, a new analgesic, 1,2′-diphenyl-2′-propionoxy-3′-methyl-4′-dimethylaminobutane (Compound 16298, Lilly), was compared to codeine phosphate and acetylsalicylic acid in blind tests. Capsules, identical in appearance, containing 50 mg. of Compound 16298, 32.5 mg. of codeine phosphate, 325 mg. of acetylsalicylic acid, or placebo powder were administered every 4 hours to patients with chronic pain. Either of two doses (1 or 2 capsules) was given for a period of 16 days before a switch was made. A "treatment series" lasted for 4 consecutive days. During this time a different one of each of the four drugs was given each day, at the same dosage level, but in randomized fashion. No other medication or manipulation was allowed during this period. The degree of pain was recorded by an observer who did not know the type of medication given. The pain score was defined as the sum of products obtained by multiplying the hours of severe pain by 3, the hours of moderate pain by 2, and the hours of slight pain by 1.

The only data selected for statistical anaylsis were from patients who received at least 3 complete series at each dose level, who reported less than 20 per cent days with no pain, and who had a greater total pain score with placebo than with codeine phosphate or acetylsalicylic acid therapy. Of 84 patients screened, only 13 met these requirements. These 13 patients had received 616 days of unbroken therapy.

Analysis of the data indicates that Compound 16298, as well as the two control drugs, showed analgesic activity. Although with all three drugs patients experienced greater pain relief at the higher dose level, they were unable to distinguish between the different drugs.

The best estimates of relative potency obtained from this experiment indicate that 80 mg. of Compound 16298, Lilly, is equivalent to 325 mg. of acetylsalicylic acid; 50 mg. of Compound 16298 is equivalent to 32.5 mg. of codeine phosphate.

67. EFFECT OF HYPOTENSION DUE TO SPINAL ANESTHESIA ON CORONARY BLOOD FLOW AND MYOCARDIAL METABOLISM IN MAN

DONALD B. HACKEL, M.D., SALVATORE M. SANCETTA, M.D., AND JEROME KLEINERMAN, M.D., CLEVELAND, OHIO

(INTRODUCED BY ROY W. SCOTT, M.D.)

Hypotension has been feared as a potential hazard to the human heart during spinal anesthesia. Any deleterious effects should depend upon a possible undue restriction of coronary flow by the hypotension, out of proportion to any decrease in myocardial oxygen demand at the diminished level of cardiac work. The present studies employ the method of coronary sinus catheterization to determine the effects of arterial hypotension due to spinal anesthesia on coronary blood flow and oxygen metabolism of the heart.

The coronary sinus of 12 patients was catheterized, and control measurements were made of coronary blood flow (N20 method) and myocardial oxygen extraction. Six patients were then given spinal anesthesia and the measurements were repeated. The level of anesthesia was "high" (above T4) in 4, and "low" in 2 patients, but all 6 patients showed a marked drop in intra-arterial blood pressure. The other 6 patients served as "double controls" to determine the variations between the two sets of observations without the production of hypotension.

In all patients given spinal anesthesia there was a drop in coronary flow parallel to the drop in mean arterial blood pressure. Nevertheless, 5 of the 6 patients showed little change, or an actual increase, in coronary sinus oxygen content. The myocardial oxygen extraction of coefficient (A-V/A) was therefore unchanged or decreased. This indicates that myocardial oxygenation was adequate for the decreased work load. The sixth patient in this series had a sudden blood pressure drop during the sampling period following the induction of spinal anesthesia. He showed an increased myocardial oxygen extraction coefficient and transient electrocardiographic subendocardial ischemic changes.

A striking contrast exists between these findings and observations during hemorrhagic shock in dogs. In both groups the blood pressure was comparably reduced, but in the dogs in hemorrhagic shock coronary flow and myocardial oxygen extraction were maintained at a relatively high level. In addition, the myocardial oxygen extraction coefficient was increased, indicating relative coronary insufficiency.

68. RELATIVE PLASMA CONCENTRATION OF TETRAZOLIUM AND PHENYLHYDRAZINE REACTING STEROIDS IN HEALTHY AND DISEASED ADULT HUMAN SUBJECTS

HARRY W. HAEGEMEIER, M.D. (BY INVITATION), SMITH FREEMAN, M.D., PH.D., AND MRS. JOANNA X. WHEELER, B.S. (BY INVITATION), CHICAGO, ILL.

Plasma from normal subjects that is extracted, washed, concentrated, and chromatogramed gives fairly constant values when color is developed on aliquots of eluate by means of the tetrazolium or phenylhydrazine reagents. In normal subjects reduction of tetrazolium usually corresponds to 30 to 40 gamma of compound F per 100 ml. of plasma. The reaction with phenylhydrazine has given normal values equivalent to 60 to 70 per cent of those obtained by the tetrazolium method. This ratio is quite constant in the normal fasting subject.

Determinations of this ratio in various disease states indicate that the per cent of phenylhydrazine reacting steroids was consistently reduced in individuals with hypoadrenal, hypopituitary, and hypothyroid function; also in patients with cor pulmonale, as well as in subjects with manifestations of acute schizophrenia. This ratio was found to be within the normal range in patients with duodenal ulcer, diabetes mellitus, rheumatoid arthritis, and in one instance of Cushing's syndrome. A low total reducing steroids and a low proportion for those reacting with phenylhydrazine usually occur together and may be temporarily increased by stimulating the adrenal gland with ACTH. Administration of 100 mg. of cortisone orally to fasting subjects will also temporarily increase the fraction reacting with phenylhydrazine. Ordinarily, the ratio returns to the initial value within four hours. It is evident from studies on various clinical and experimental states that some of the substances reacting with both reagents are not adrenal in origin.

69. FECAL UROBILINOGEN EXCRETION IN HEMOLYTIC ANEMIA: COMPARISON WITH RESULTS OF AVERAGE RED CELL LIFE DETERMINED BY RADIOCHROMIUM RED CELL TAGGING

PAUL S. HAGEN, M.D., AND ROSE M. MACDONALD, B.S., MINNEAPOLIS, MINN.

(INTRODUCED BY C. J. WATSON, M.D.)

Until the advent of red cell tracer methods, fecal urobilinogen excretion was the only practical measurement of the rate of red cell destruction in hemolytic anemias. However, it has been generally appreciated that really quantitative results were not obtained because of certain variables in fecal urobilinogen excretion. Further doubt has been raised by glycine-N^{15} studies which have shown that there are sources of bile pigment other than the hemoglobin of adult circulating red cells. The present study compared the per diem fecal urobilinogen excretion measured in four-day stool collections with the urobilinogen potentially available from the daily destruction of circulating red cells. The potential urobilinogen was calculated from the average circulating hemoglobin mass and the average life of the red cells. Radiochromium-labeled red cells were used to measure the average red cell life. An aliquot of the patient's blood was incubated in vitro with $Na_2Cr^{51}O_4$ solution and reinjected into the patient. The radioactivity of sequential blood samples was measured in a well-type scintillation counter.

Fecal urobilinogen "recovery" (observed fecal urobilinogen/potential urobilinogen $\times$ 100) figures were as follows: four patients with acquired hemolytic anemia, 79, 87, 51, and 56 per cent (after splenectomy the figure for the last patient was 57 per cent); in one patient with paroxysmal nocturnal hemoglobinuria, 41 per cent; in two patients with hemoglobin-C disease, 90 and 105 per cent; in two patients with hereditary spherocytosis, 113 and 57 per cent. The last figure was obtained twenty-one months after a twenty-five-day course of oxytetracycline. Comparison with fecal urobilinogen excretion before the antibiotic gave a "recovery" of 113 per cent. The patient was in the same clinical state during both periods of study. In marked contrast to the hemolytic anemia patients, one patient with pernicious anemia in severe relapse had a fecal urobilinogen "recovery" of 675 per cent.

These comparative studies showed that in hemolytic anemia there was a reasonable correlation between fecal urobilinogen excretion and the rate of destruction of circulating red cells. These studies confirm the clinical usefulness of fecal urobilinogen in the diagnosis of hemolytic anemia.

70. INFLUENCE OF SMALL DOSES OF ANTIBIOTICS ON THE WEIGHT BEHAVIOR OF YOUNG MALES

THOMAS H. HAIGHT, M.D., AND WILLARD E. PIERCE, GREAT LAKES, ILL.

(INTRODUCED BY CLAYTON G. LOOSLI, M.D.)

During a study on the effects of antibiotic prophylaxis upon the immune response, serial measurements were also made of the nude weight of the Navy recruits being observed. These 330 subjects had been selected upon their arrival, carefully interviewed, and assigned at random to one of three treatment groups. For seven weeks each man received once daily an identical-appearing capsule which contained one of the following preparations: (1) 250 mg. of chlortetracycline (Aureomycin); (2) 100,000 units of buffered procaine penicillin; or (3) a placebo. All subjects were weighed initially and again four and seven weeks later. The relative body weights (actual weight

expressed as the per cent of a standard weight for the age and height) were also calculated. The individual and group changes in actual and relative body weight were tabulated, and it was found that a highly significant difference existed between the weight gain in the antibiotic groups and the placebo group ($P < 0.05$). Those who had received either antibiotic had about equal average total gains (Aureomycin group 4.8 pounds, penicillin group 4.1 pounds), but greater average gains than those who received the placebos (2.7 pounds).

Because this observation was unexpected, a further controlled experiment was conducted. The populations and methods were identical to the first study, except that no capsules were administered. Also, whereas the first study had been conducted during the winter, the second was during the late spring and early summer. Serial nude weights were recorded for three artificially randomized groups, and it was apparent that there were no group-to-group differences, either in actual or relative body weight changes (average total gain: Group 1, 0.7 pound; Group 2, −0.2 pound; Group 3, 1.2 pounds). It was felt that this experiment established that the group-to-group differences during the period of antibiotic prophylaxis were not likely to be due to chance alone.

These findings are of great interest in view of the known specific nutritional effects of the antibiotics in children and animals, but it was surprising to find that young males in a final slow phase of growth might have their weight behavior influenced by administration of small doses of either of two antibiotics for seven weeks. These observations are preliminary, and it is suggested that further experiments be conducted along these lines.

71. STUDIES IN THE MODE OF ACTION OF ANTIBIOTICS IN BACTERIAL ENDOCARDITIS: AN INVESTIGATION OF THE TIME REQUIRED FOR PENICILLIN TO KILL STREPTOCOCCI FROM PATIENTS TREATED SUCCESSFULLY AND PATIENTS WHO RELAPSED

MORTON HAMBURGER, M.D., AND JUDITH CARLETON (BY INVITATION),
CINCINNATI, OHIO

The reason why certain patients infected with penicillin-sensitive *Streptococcus viridans* can be successfully treated by a given dose of penicillin whereas other patients relapse following the use of the same treatment regimen has never been explained. The minimal inhibiting concentration of penicillin, as determined by conventional serial dilution tests, lies within the same narrow range for strains from both types of patients. However, the work of Eagle, Hunter, and others has suggested the possibility that the actual time required for penicillin to produce its bactericidal action may be longer for streptococci from patients who relapsed than for strains from patients who were successfully treated.

In order to test this possibility, the bactericidal rate of penicillin was measured against 5 strains of penicillin-sensitive *Streptococcus viridans* from patients who were successfully treated and against 5 strains from 3 patients who relapsed one or more times. Large, medium, and small numbers of bacteria were inoculated into separate flasks containing no penicillin, 50 μg per milliliter, 2 μg per milliliter, 0.5 μg per milliliter, and 0.05 μg per milliliter. The flasks were incubated at 37° C. Aliquots were removed at the end of each twenty-four hours and subcultures made, using pour plates and broth cultures. Subcultures were made daily until the flasks were sterile.

The results demonstrated no difference between the penicillin killing times for the "relapse" strains and "nonrelapse" strains. Furthermore, the time

required to sterilize a given culture was the same at the higher as at the lower concentrations of penicillin investigated. However, large bacterial inocula were killed much more slowly than small ones, regardless of the origin of the strain or of the concentration of penicillin.

It is concluded that differences in penicillin-killing time among strains of penicillin-sensitive streptococci are not important in determining the success of treatment in bacterial endocarditis, though the number of bacteria present in the lesion may be very important indeed.

72. THE EFFECT OF ACTH ON A HEMOLYTIC MECHANISM IN TRICHINOSIS

HENRY E. HAMILTON, M.D., ELMER L. DEGOWIN, M.D., AND RAYMOND F. SHEETS, IOWA CITY, IOWA

A 21-year-old man with a severe illness characterized by diarrhea, periorbital edema, diplopia, muscle pain and rigidity, high fever, and delirium was the subject of this study. Flame shape hemorrhages were noted in the eyegrounds and a tic-tac cardiac rhythm was present. The electrocardiogram showed changes characteristic of myocardial disease. The eosinophils made up 48 per cent of the differential count. The sedimentation rate was slightly elevated. A muscle biopsy demonstrated a high concentration of live larvae of *Trichinella spiralis*. The clinical course was of such violent nature that recovery from the illness was doubtful. On the seventeenth day of illness a total of 80 mg. of ACTH was given and continued with varying dosage for 28 days. A marked improvement of the systemic reaction occurred within 48 hours and the rectal temperature dropped from 104° to 99.0° F. Recovery gradually occurred during the next three months. The reticulocyte count remained slightly elevated, the total erythrocyte count varied between 4 millions and 5 millions per cubic millimeter, and the eosinophilia persisted during the recovery period.

A transfusion of biologically tagged erythrocytes was given on the sixteenth day of illness and the tagged cells were enumerated thereafter by the Ashby technique of differential agglutination. The tagged cells disappeared from the circulation at an accelerated rate during 80 days of observation. The random rate of loss of tagged erythrocytes was 0.68 per cent per day (DeGowin et al., J. Clin. Invest. 33: 163, 1954). The rate of hemolysis of the tagged cells was not altered by ACTH therapy. If it is assumed that the patient's cells were being destroyed at the same rate as the biologically tagged cells, then erythropoiesis was increased during the period of illness to maintain a normal erythrocyte level.

Random destruction of tagged red cells persisted in spite of ACTH therapy in a patient seriously ill with trichinosis. ACTH, however, did change the course of the disease and in all probability was responsible for recovery.

73. STUDIES ON THE MECHANISM OF A DRUG-INDUCED HEMOLYTIC ANEMIA

JOHN W. HARRIS, M.D., CLEVELAND, OHIO

(INTRODUCED BY CHARLES H. RAMMELKAMP, JR., M.D.)

An acute hemolytic episode developed in a 24-year-old man, which was manifested by rapidly developing anemia, hemoglobinemia, methemalbuminemia, bilirubinemia, hemoglobinuria, reticulocytosis, spherocytosis, and increased erythrocyte osmotic and mechanical fragility; his erythrocytes gave

a positive test in a 1:2,048 dilution of Coombs serum. This hemolytic process occurred during retreatment of schistosomiasis with Fuadin.

A factor was demonstrated in the serum of this patient that strongly agglutinated his own or normal erythrocytes; it was active only when Fuadin was present in the reacting system. Normal cells exposed to this serum became Coombs positive, but only if Fuadin had been added. The agglutinin was nondialysable, not significantly influenced by pH or temperature alterations; it remained active in heated serum. Other antimony compounds and catechol did not activate the agglutinin; however, the addition of sodium catechol disulfonate potassium resulted in agglutination demonstrating that the entire Fuadin molecule was not necessary. Initially the Fuadin-requiring agglutinin was active in a 1:1,024 dilution of the patient's serum but the titer gradually fell to one-sixteenth over a period of sixty days.

Passive transfer of the agglutinin was effected by administration of 300 ml. of the patient's plasma to a hematologically normal recipient. Five days after the plasma infusion the recipient was given Fuadin intravenously. Although hemoglobinemia and bilirubinemia did not develop and the erythrocyte osmotic and mechanical fragilities were not significantly altered, gross erythrocyte agglutination did occur; the recipient's cells became Coombs positive and the titer of circulating agglutinin diminished. It was present for a total of twenty-six days in detectable amounts.

It is believed that this is the first reported instance of acquired hemolytic anemia secondary to drug administration in which a serum factor requiring the presence of the drug for agglutinating activity has been demonstrated.

74. THE VARIED PATTERNS OF WATER AND SODIUM DIURESIS DURING ACTH THERAPY OF THE NEPHROTIC SYNDROME

G. H. Heidorn, M.D. (By Invitation), F. R. Schemm, M.D., and John A. Layne, M.D., Great Falls, Mont.

Nine patients with the nephotic syndrome receiving ACTH were studied in metabolic detail. This report is concerned only with the water and sodium balance aspects of their study. Seven of these nine responded well to therapy even though the dosage of ACTH used was lower than those usually recommended. Estimated weight gain or loss was calculated from sodium balance data. Estimated weight changes were then compared to actual weight changes before, during, and after ACTH administration.

Varied patterns of water and sodium diuresis were observed. Spontaneous weight loss began during ACTH administration in three patients. There was a weight gain during ACTH therapy and a weight loss following its discontinuance in two other patients. The remaining patients diuresed during and after ACTH with the aid of mercurial diuretics. Spontaneous diuresis of water in excess to sodium occurred in two patients while receiving ACTH. One patient exerted sodium in excess to water during this period. The two patients who gained weight during hormone therapy retained water in excess to sodium. Diuresis following the discontinuance of ACTH consisted of sodium in excess to water (three patients), water in marked excess to sodium (one patient following the onset of menstruation), and proportionate amounts of water and sodium (one patient).

In addition, renal tubular sensitivity to mercurial diuretics is often regained during and after the administration of ACTH. The qualitative effect of mercurials likewise is altered by this hormone. Water diuresis in excess to sodium following an injection of mercury was observed before or early in

the course of ACTH (three patients); whereas, sodium was lost in excess to water later in the course or after ACTH was discontinued (three patients).

The great variability of water and sodium diuresis during ACTH therapy is presented to stress the physiologic complexities of this disease; as well as recording what might be expected during therapy. The changing pattern of mercurial action when ACTH is administered is of practical worth and also emphasizes the possible direct effect of ACTH or its hormonal associates from the adrenal cortex on renal tubular enzyme systems.

75. RENAL FUNCTIONAL STATUS IN PATIENTS WITH ACROMEGALY

Ben I. Heller, M.D., Ralph Eugene Smith, M.D. (By Invitation), and Robert I. Lubin, M.D. (By Invitation), Minneapolis, Minn.

That the kidneys frequently participate in the visceromegaly which forms an integral part of clinical acromegaly has long been appreciated. In addition, the renotrophic action of anterior pituitary extracts has been documented in certain animals.

Renal functional changes associated with anterior pituitary extract administration to dogs have been detailed and somatotrophin has been indicted as the responsible factor. These functional changes include doubling of PAH clearance and marked increases in inulin clearance and TmPAH after ten to fourteen days of growth hormone administration. Data concerning the renal functional status of patients with uncomplicated clinical acromegaly have not been published and this study is an effort to define, functionally, this group.

Studies utilizing the clearance and saturation methods devised by Smith, Goldring, Chassis, and co-workers were conducted on five male patients with clinically apparent acromegaly. The effective renal plasma flow and maximal tubular excretory capacity were measured by the use of para-amino hippurate and the glomerular filtration rate was measured by the clearance of inulin. Considerable difficulty in obtaining adequate tubular saturation as indicated by the load : T_{PAH} ratio was encountered in spite of an increase in both the priming dose and sustaining dose of PAH, the latter to 400 mg. per minute.

The studied group is characterized by augmented renal plasma flows with correspondingly greater increases in glomerular filtration rates leading to high filtration fractions and, in addition, abnormally high tubular excretory capacities.

76. PROPERDIN: A SPECIFIC HEMOLYTIC FACTOR IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Carl F. Hinz, Jr., M.D. (By Invitation), William S. Jordan, Jr., M.D., and Louis Pillemer, Ph.D. (By Invitation), Cleveland, Ohio

With the Technical Assistance of Livia Blum (By Invitation)

Similarities were pointed out earlier between the inactivation of the third component of human complement (C'3) by yeast cell stroma (zymosan), and the hemolysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH). Both phenomena are pH and Mg^{++} ion dependent, and both require the presence of serum complement although neither requires the presence of specific antibody. This similarity was further emphasized by the demonstration that stroma from PNH erythrocytes, like zymosan, removes C'3 from serum at 37° C.

As a necessary factor for the zymosan inactivation of C'3, Pillemer and his colleagues have recently identified and isolated properdin, a new serum euglobulin with a molecular weight of about 1,000,000, which is distinct from complement and the components of the clotting system. Properdin combines with zymosan at 17° C. in the presence of Mg^{++} and complement, and is concerned with natural immune phenomena.

The following observations indicate that properdin is also a specific factor in the PNH hemolytic system: (1) normal serum from which properdin has been removed at 17° C. by zymosan (RP serum) is completely inactive against PNH erythrocytes. This RP serum contains all components of complement and of the clotting system, and is fully active in specific immune hemolytic systems including anti-A and paroxysmal cold hemoglobinuria; (2) purified properdin from human, cow, or hog serum restores the hemolytic activity of RP serum for PNH erythrocytes although properdin alone is not lytic; (3) the PNH lytic activity of serum parallels the properdin titer, and sera with no properdin fail to lyse PNH erythrocytes; (4) the augmented hemolysis of PNH erythrocytes that is noted on addition of thrombin is proportional to the amount of properdin contained in thrombin preparations.

These data indicate that properdin, a normal serum protein, is a specific factor in the destruction of the abnormal PNH erythrocyte. It requires Mg^{++} and complement for activity, but is separate from both complement and the clotting system.

It is possible that properdin combines with the stroma of PNH erythrocytes, participating in their destruction in a manner analogous to its role as a natural immune substance in virus neutralization and bacterial destruction.

77. THE ACTION OF ALCOHOL ON GASTRIC FUNCTION

Basil I. Hirschowitz, M.D. (By Invitation), S. W. Hartwell, Jr., M.D. (By Invitation), and H. Marvin Pollard, M.D., Ann Arbor, Mich.

Because of the almost universal habit of using alcohol as an appetizer, we investigated the effects of oral alcohol on gastric function and further investigated the mechanism of action of alcohol on the stomach.

The effects of one to four 40 ml. doses of whiskey orally on gastric secretion and motility (by balloon-kymography) during a four-hour period was determined in eight subjects. There were no effects on motility or volume of gastric juice. Acid concentrations were increased in 13 of 22 studies. This suggests that apart from the traditional objection, the standard alcohol test meal is also unreliable.

In over 50 studies with intravenous alcohol given in the third half-hour of a four-hour gastric analysis, acid production was invariably stimulated to at least twice the control levels. There was no significant effect on volume or gastric pepsin. Eight per cent alcohol was more effective in stimulating acid production than 4 per cent, but no less than 16 per cent, indicating a threshold at about the 8 per cent level.

To determine the site of action of alcohol, 250 ml. of 8 per cent alcohol was given intravenously and this reversed the depressant effects of subcutaneous hexamethonium given previously, in 7 of 8 studies. Alcohol, therefore, acts peripherally. To further define this, intravenous alcohol given one-half hour after 1.0 to 1.5 mg. atropine sulfate subcutaneously neither delayed nor reversed the expected depressant effects of the atropine on gastric acid or volume. Atropine given after alcohol reversed its stimulant action. Alcohol,

therefore, probably acts peripherally at the synaptic level, rather than by any histamine-like action, as is at present believed, since the latter would not be prevented or reversed to that degree by atropine.

Simultaneous estimations of blood and gastric alcohol levels showed that the peak of blood alcohol concentration was reached at the end of the alcohol infusion, while the peak concentration in the gastric juice occurred thirty minutes later and was in almost every instance higher than any blood levels. This suggests an active excretion of alcohol into the stomach, and this may play a part in the pathogenesis of alcoholic gastritis.

78. A HEMATOCRIT CORRECTION FOR TRAPPING OF PARA-AMINOHIPPURIC ACID: ITS USE IN ESTIMATION OF RENAL BLOOD FLOW

MELBA HOLDER, CLYDE SCHOENFELD, AND JAMES SCHIEVE, M.D.,
COLUMBUS, OHIO

(INTRODUCED BY B. K. WISEMAN, M.D.)

In the estimation of effective renal blood flow (ERBF) from effective renal plasma flow (ERPF) a correction for the amount of para-aminohippuric acid (PAH) remaining in the red cell column of a centrifuged hematocrit is usually disregarded. Rarely, a correction of 5 per cent is used, chosen from widely varied (2.5-12 per cent) values available from experience, mostly with Evans blue dye. This factor can best be determined by using PAH, as the critical factor is not the amount of plasma trapped between red cells but the amount of PAH remaining. Whether PAH is in trapped plasma or loosely attached in or on red cells of the red cell column is inconsequential for estimation of ERBF.

PAH has been completely recovered from plasma (99+ per cent in each of ten samples). It is virtually completely recovered after washing whole blood with saline (97+ per cent from six blood samples). These findings are in agreement with those reported by Smith. If PAH is attached to red cells rather than in a trapped plasma space, the attachment appears a loose one.

To detect "trapping" of plasma or loss of PAH in the Wintrobe hematocrit, the technique of dilution of known amounts of PAH added to known volumes of whole blood was used. The dilution of PAH in the plasma of the blood sample thereby allowed estimation of a dilution hematocrit. The dilution hematocrit was compared to the Wintrobe hematocrit of its paired blood sample. Wintrobe hematocrits were centrifuged for at least sixty minutes at 3,000 r.p.m. with a radius of 14.5 cm. (1,400 RCF).

The results show the centrifuged hematocrit was consistently higher than dilution hematocrit (average 8.8 per cent with a range of 7.4 to 9.9 per cent). The Wintrobe hematocrit readings were obtained from ten patients and ranged from 29 to 84 per cent.

It is suggested an 8.8 per cent correction of the Wintrobe hematocrit be used in estimation of ERBF. Changes in ERBF in absolute numbers will be much greater as the hematocrit increases and will be significantly less in anemia. For example, this factor corrects estimated ERPF from 1,143 to 758 ml. per minute in a patient with secondary polycythemia who had decreased ERPF, while in a patient with anemia it changes ERBF from 615 to 601 ml. per minute.

79. NEUTRAL POLYSACCHARIDE STAIN IN NEEDLE BIOPSY SPECIMEN OF THE LIVER

W. Joel, M.D., E. M. Schneider, M.D., and M. L. Clark, M.D.,
Oklahoma City, Okla.

(Introduced by Robert H. Bayley, M.D.)

In twenty-five consecutive needle biopsies of the liver performed on patients with various diseases, both hematoxylin and eosin and neutral polysaccharide (glycogen) stains were performed. The polysaccharide stain is technically no more difficult than the routine hematoxylin and eosin stain and was found to increase the diagnostic value of the biopsy specimen. Through the use of this technique, it was possible to determine the glycogen content of hepatic cells and to permit an estimate of the degree of hepatocellular dysfunction in several cases where routine hematoxylin and eosin stains failed to reveal any abnormality. The presence of glycogen in hepatic cell nuclei, which is an abnormal finding, was demonstrated only by this technique. Finally, the contrast between nonglycogen and glycogen containing cellular material was such that even slight degrees of fibrosis were readily discerned by the use of the polysaccharide stain.

All stains were performed on both alcohol-fixed and formalin-fixed tissue—thus permitting the polysaccharide stain to serve as an estimate of the quantity and stability of the intracellular glycogen. It is felt that the routine use of neutral polysaccharide stain in the study of needle biopsy specimens of the liver broadens the usefulness of this already valuable diagnostic technique.

80. THE RECORDING OF THE ARTERIAL BLOOD PRESSURE AND ARTERIAL CIRCULATION

Carl A. Johnson, M.D., Chicago, Ill.

These studies were made on a recording oscillometer which makes a timed, calibrated optical recording of the pulse wave anywhere in the extremities. The physical characteristics of the instrument are: (1) a natural frequency of the recording droplet in the range of 100,000; (2) a lag of 0.006 seconds; (3) an accurate responsiveness to all human heart rates; (4) a built-in sensitivity reduction of 10:1 for adults and 5:1 for infants; (5) an overshoot which is corrected for by a constant "K." An artificial arm, constructed for testing the accuracy of the oscillometer, by which it is possible to control diastolic pressure, heart rate, and overshoot will be described. An illustration of an artificial pulse wave produced on this instrument comparable to the human pulse wave will be shown. The systolic blood pressure was measured by the double cuff method, and the manner of making this recording will be described and illustrated. The diastolic pressure was recorded at a point determined by established physical principles for elastic systems, and will be described and illustrated. The arterial circulation was recorded on a calibrated recording of the pulse wave and is expressed in milliliters per beat or per minute. Data will be presented indicating that this figure can be determined with an accuracy which is thought by the author to be within the range of plus or minus 3 per cent. The formula developed for correction of overshoot and heart rate will be described. A typical recording of the arterial blood pressure above the elbow and above the knee, as well as recordings of the arterial circulation above the elbow, below the elbow, above the knee, below the knee, and at the ankle will be shown on a lantern slide.

81. BODY COMPOSITION AND OXYGEN CONSUMPTION OF UNDERWEIGHT, NORMAL, AND OVERWEIGHT WOMEN

LOUIS C. JOHNSTON, M.D., CHICAGO, ILL.

(INTRODUCED BY FORD K. HICK, M.D.)

Basal oxygen consumption, antipyrine space, and inulin space were determined on seventeen normal women whose weight ranged from 40 kg. (87 pounds) to 186 kg. (410 pounds) or from 60 to 284 per cent of standard weight. Antipyrine space (total body water) was determined by the single injection technique of Soberman and associates (J. Biol. Chem. 179: 31, 1949) and was used to calculate the percentage of body fat and lean body mass, i.e., nonfatty body weight, by the method of Pace and Rathburn (J. Biol. Chem. 158: 685, 1945). Inulin space (extracellular water) was measured by the constant infusion technique of Guadino (Proc. Soc. Exper. Biol. & Med. 68: 507, 1948) and with antipyrine space was used to compute cell mass by the method of McCance and Widdowson (Proc. Roy. Soc., London sB 138: 115, 1951). Basal oxygen consumption was measured at the bedside by a clinical machine.

The basal oxygen consumption was found to correlate equally well with surface area, lean body mass, and cell mass ($r = .91, .94$, and $.92$, respectively). This similarity of correlations is explained by the high correlation of surface area with both lean body mass and cell mass ($r = .91$ for both). The body fat in this series ranged from 7 to 88 kg., or from 17 to 57 per cent of body weight. Both the percentage and the weight of fat was found to increase with increases of body weight above standard. However, this increase was found to be decremental, or progressively decreasing; per cent fat $= -70.0 + 53.3$ log standard weight. Cell mass was found to decrementally decrease according to the formula: per cent cell mass $= 118.2 - 36.1$ log standard weight. Therefore, overweightness of the women in this series is attributed to accumulation of both fat and cell mass, and the average overweight tissue was found to consist of 67 per cent fat, 24 per cent cell mass, and 6 per cent extracellular fluid.

Extracellular fluid was found to correlate better with total body water than with body weight. The percentage of total body water that is extracellular correlated neither with the degree of overweightness or the percentage of fat present.

82. THE SERUM LIPID PATTERN IN HYPOTHYROIDISM, HYPERTHYROIDISM, AND CORONARY ARTERY DISEASE

RICHARD J. JONES, M.D., HOWARD CORBUS, M.D. (BY INVITATION), AND LOUIS M. COHEN, M.D., (BY INVITATION), CHICAGO, ILL.

Sera from 17 patients with definite hypothyroidism were compared with the sera from 25 patients with hyperthyroidism, and 17 patients with coronary artery disease or essential familial hypocholesteremic xanthomatosis matched with the myxedema group as to sex, serum cholesterol, and age. Each serum was analyzed chemically for total lipid, cholesterol, and phospholipid and analyzed physically by the heavy density ultracentrifugal technique of Lewis and Page. There was a significant increase in all the lipid fractions, except for the alpha lipoprotein, in myxedema as compared to hyperthyroidism. The mean beta lipoprotein was increased almost threefold, the chemical fractions twofold of that seen in hyperthyroidism.

The lipid pattern in myxedema was found to be almost identical to that in the matched group with coronary artery disease and comparable hyperlipemia. The one exception was a smaller beta lipoprotein concentration for a given level of serum cholesterol in the myxedema group. This supplies further evidence to suggest a different mechanism in the development of hyperlipemia in myxedema and in atherosclerosis.

83. THE SEROLOGIC EPIDEMIOLOGY OF RESPIRATORY AGENTS ISOLATED IN HeLa CELL CULTURES

William S. Jordan, Jr., M.D., George F. Badger, M.D. (By Invitation), John H. Dingle, M.D., Harold S. Ginsberg, M.D., and Sidney Katz, M.D. (By Invitation), Cleveland, Ohio

Four different types of respiratory agents, presumably viruses, have recently been isolated in tissue cultures and can be identified by specific serum neutralization of their cytopathogenic effects in HeLa cell cultures. One of these agents, RI-67 (Hilleman and associates, Proc. Soc. Exper. Biol. & Med. 85: 183, 1954), has been causally related to a grippelike illness of military recruits termed ARD, and neutralization tests suggest that it was responsible for both epidemic and endemic illnesses so classified during World War II. Three agents, AD Types 1, 2, and 3 (Rowe and co-workers, Proc. Soc. Exper. Biol. & Med. 84: 570, 1953), were isolated from normal adenoid tissue, but their relation to disease is obscure.

Of the 155 sera obtained in the spring of 1954 from a civilian population, 29 (18.7 per cent) contained neutralizing antibodies for RI-67. None of the 73 children tested had antibodies; 17 per cent of the mothers and 53.7 per cent of the fathers had antibodies. Their presence was not correlated with previous military service.

An agent, termed JL virus, immunologically similar to AD Type 3, was isolated from a patient with a grippelike illness. Neutralizing antibodies in his serum increased 64-fold against his own and the Type 3 agent and fourfold against AD Type 1. Paired sera from 58 adults with respiratory illnesses observed between 1947 and 1954 were tested against the RI-67 and JL agents. Neutralizing antibodies were present in the acute sera in 34 per cent for RI-67 and in 36 per cent for JL. Twenty of these same sera were tested against AD Types 1 and 2; 40 per cent contained antibody for Type 1, 40 per cent for Type 2. Eleven individuals developed increases in titer. One patient with nonstreptococcal exudative pharyngitis developed a 16-fold rise to JL and fourfold rises to RI-67 and AD2. Two of the three rises against RI-67 occurred in persons working with this agent. Six rises were detected with the recently isolated JL agent, three with AD2, and one with AD1.

Thus, RI-67 has produced few infections in Cleveland in recent years. The JL agent appears to be associated with syndromes classified as ARD and nonstreptococcal pharyngitis.

84. AMINOACIDURIA FOLLOWING TOTAL BODY IRRADIATION IN THE HUMAN BEING

Elaine J. Katz, M.A., and Robert J. Hasterlik, M.D., Lemont, Ill.

(Introduced by Austin M. Brues, M.D.)

Following the accidental exposure of four laboratory workers to total body irradiation with gamma rays and neutrons at levels between 25 and 180 rep

(roentgens equivalent physical), total daily urinary amino acid excretion increased to ten times normal. The quantities of individual amino acids excreted varied from two to twenty times normal amounts.

In all cases, abnormal excretion in both number and quantity of amino acids occurred as early as twelve hours following the initial exposure, and persisted as long as two weeks. Glycine was excreted to the greatest extent, but also of note was the marked increase over normal excretion of cystine, taurine, glutamic acid, aspartic acid, and hydroxyproline. In agreement with findings in previous radiation accidents, a peak in excretion was reached in all patients on the sixth day after exposure.

Complete hematological studies on these patients indicate that the rate and magnitude of the drop in absolute lymphocyte count and total white count is roughly proportional to the radiation exposure level and is useful as an indicator of the magnitude of the radiation exposure, but aminoaciduria may be a significantly more sensitive early indicator of radiation overexposure.

It is believed that aminoaciduria after irradiation may be related to the following: (1) decreased oxidation and utilization of amino acids by the liver and other tissues; (2) partial inhibition of amino acid oxidation in the kidney; (3) adrenal response to a stress situation; (4) increased protein (tissue) breakdown. In view of the response to an exposure of 25 rep, it is believed that increased protein breakdown is not the major factor in producing the aminoaciduria found in these patients.

85. TREATMENT OF SPONTANEOUS (ESSENTIAL?) HYPERTENSION IN DOGS WITH RENIN AND ANTIRENIN

J. I. Katz, B.S., M.D. (By Invitation), J. Skom, M.S., M.D. (By Invitation), and G. E. Wakerlin, Ph.D., M.D., Chicago, Ill.

In all respects thus far studied, so-called spontaneous hypertension in dogs resembles early essential hypertension in man, and knowledge of the pathogenesis of the former may help elucidation of the latter. In view of the antihypertensive effect of antirenin produced by a course of intramuscular injections of crude or semipurified hog renin and the antihypertensive effect of passively transferred antirenin to hog renin, in dogs with experimental renal hypertension, we studied the effects of courses of crude and semipurified hog renins and of the passive transfer of antirenin in spontaneous hypertensive dogs. Three spontaneous hypertensive dogs were treated with crude hog renin and six with semipurified hog renin from cortex in doses of 2.5 to 20 D. U. (dog units) per kilogram daily and intramuscularly for four to nineteen months. These dogs showed highly significant antihypertensive effects which were well correlated with the plasma antirenin titer. Similarly prepared control tissue extracts were without antihypertensive effects in four spontaneous hypertensive dogs. Five spontaneous hypertensive dogs were passively immunized on two separate occasions with high titer homologous antisera containing antirenin to semipurified hog renin. Significant antihypertensive effects were obtained. Control experiments with low titer antisera were ineffective. The passively produced antirenin titers not only correlated well with the antihypertensive effect but the effective titers were similar to those produced by injections of semipurified hog renin into spontaneous hypertensive dogs. Control normotensive dogs, including two with high normotensions, show no depressor effect from a course of crude or semipurified hog renin with antirenin production or from passive immunization with antirenin.

These results suggest a renal factor operating through renin or a closely related renal protein in the pathogenesis of spontaneous hypertension in the dog and offer encouragement to those who are presently attempting to alter the antigenicity of hog renin so that antirenin to hog renin will neutralize human renin. If this attempt is successful, study of such modified hog renin in essential hypertension may determine what percentage, if any, of these patients have their hypertension on a renal, renin basis.

86. A COMPARISON OF THE METABOLIC EFFECTS OF A SINGLE INTRAVENOUS INFUSION OF HYDROCORTISONE WITH ITS PLASMA LEVELS

Luther W. Kelly, Jr., M.D., Richard P. Levy, M.D., Katherine L. Sydnor, M.D., and William McK. Jefferies, M.D., Cleveland, Ohio

(Introduced by Max Miller, M.D.)

Since recent studies have indicated a rapid turnover of hydrocortisone in human beings, a study was undertaken to correlate the onset and duration of the metabolic effects of a single injection of this steroid with its disappearance from the blood stream. One hundred milligrams was infused intravenously within 30 minutes into a normal adult male and into one subject with Addison's disease, the latter being studied while on maintenance hydrocortisone therapy and again 48 hours after therapy withdrawal. Blood levels of free hydrocortisone were determined by the method of Sweat, and free and conjugated 17-hydroxycorticosteroids in the urine by the method of Sandberg and associates.

In all experiments maximum blood steroid levels were reached at the completion of the infusion, with a rapid fall to normal levels by the eighth hour. In the Addisonian, as would be expected, further falls to subnormal levels occurred, reaching the lowest values by the twenty-fourth hour in each instance. Urinary steroid excretion was similar in all experiments, totaling approximately 30 per cent of the dose, the majority being excreted within 8 hours in a conjugated form.

Outstanding metabolic effects included: (1) a depression of circulating eosinophils for at least 8 hours; (2) increased potassium excretion which was complete by the fourth hour; (3) sodium retention, which was not evident until after the fourth hour but lasted through the first 24 hours; (4) relative loss of nitrogen, water, and creatinine in the first 4 hours. Electrolyte changes could not be explained by changes in water excretion or creatinine clearance. In general, the most striking changes occurred in the Addisonian when off therapy, but differences were quantitative rather than qualitative. It is evident that some of the metabolic effects of hydrocortisone are complete before the blood steroid levels return to normal, whereas others persist beyond this time.

87. THE PERSISTENCE OF INSULIN SENSITIVITY IN ACUTE ADRENAL HYPERACTIVITY IN MAN

Margaret Kessler, M.D. (By Invitation), and Harvey C. Knowles, Jr., M.D., Cincinnati, Ohio

Previous experiments by Fraser and associates, Conn, and Ingle have suggested that the hyperglycemia occurring in adrenal cortical hyperactivity may in part be due to insulin insensitivity. However, Zimmerman, employing sub-

jects with chronic illness, presented evidence that prolonged ACTH administration resulted in no significant decrease in insulin sensitivity. Furthermore, Engel could elicit no insensitivity in acute experiments using cortisone. Because of the inconsistency in the above observations, experiments were carried out to observe further the effects of acute adrenal hyperactivity on insulin action in normal man.

Fasting insulin tolerance tests were made on 8 normal subjects aged 23 to 37 years. Each subject was studied in the normal state, and at a later date following intravenous administration of ACTH. In the latter experiments 20 mg. of ACTH were infused over an 8-hour period, followed by 5 mg. over the ensuing 3 hours while the tests were performed. Insulin free of hyperglycemic factor was injected intravenously (0.1 unit per kilogram) and blood samples were collected at 0, 30, 60, 90, 120, and 180 minutes for determinations of sugar, serum phosphorus, and potassium. Curves were plotted to express actual mean deviations from the initial sample.

In the control experiments, 30 minutes after insulin the mean fall of blood sugar was 54 mg., phosphorus 1.36 mg., and potassium 0.62 meq. In the ACTH experiments, after insulin the mean fall of blood sugar was 47 mg., phosphorus 0.64 mg., and potassium 0.62 meq. The blood sugar response to insulin was not significantly different in the two experiments. The decreased fall of phosphorus in the ACTH group may be related to the initial lowering of serum phosphorus due to ACTH itself. Although the concentration of potassium fell the same in both groups, it rose more rapidly in the ACTH group.

It is concluded that acute adrenal cortical hyperactivity results in no significant change in insulin sensitivity. This observation may give further support to the hypothesis that metabolic alterations in acute stress are not necessarily a consequence of adrenal cortical hyperactivity.

88. KIDNEY CIRCULATION IN COARCTATION OF THE AORTA

WALTER M. KIRKENDALL, M.D. (BY INVITATION), JAMES W. CULBERTSON, M.D., AND JOHN W. ECKSTEIN, M.D. (BY INVITATION), IOWA CITY, IOWA

There has been considerable unresolved controversy concerning kidney hemodynamics in coarctation of the aorta. Some investigators postulate that renal ischemia results from the lower mean pressure in the abdominal aorta and that Goldblatt type systemic arterial hypertension follows. Others claim that hypertensive and renal hemodynamic changes result simply from mechanical constriction of the aorta.

We have studied 14 coarctation patients by standard renal plasma clearance techniques. Group I (8 patients) showed low normal renal plasma flow (RPF), normal glomerular filtration rate (GFR), normal or only slightly increased filtration fraction (FF), no significant increase in afferent arteriolar resistance (R_A) or venular resistance (R_V), and only a very questionable increase in net efferent arteriolar resistance (R'_E) or total renal vascular resistance (R). Group II (2 patients without heart failure) showed moderate decrease in RPF; great increase in FF; and selective increase in R_A, R'_E, and R_V. Group III (4 patients in congestive heart failure) had moderate decrease in RPF; significant increase in FF; and generalized increase in all components of R, with R_V and R'_E exerting a relatively greater proportion of the total effect.

General hemodynamic observations made on 10 of these 14 patients—including measurements of cardiac output (MVCO), hepatic blood flow, and splanchnic and general systemic arterial resistance—are consistent with this classification. Group I, comprising the majority of our patients, showed in-

creased MVCO, low or normal systemic resistance, decreased splanchnic resistance, and a renal hemodynamic pattern unlike that of essential arterial hypertension or unilateral kidney disease. Group II (the smallest) showed normal MVCO, increased systemic resistance, and a renal hemodynamic pattern similar to that of essential hypertension. Group III presented general and regional hemodynamic pictures characteristic of congestive heart failure.

Knowledge of general hemodynamics is important for rational interpretation of renal circulatory abnormalities. Recognition of these three cirulatory states and their significance may do much to dispel the confusion surrounding kidney hemodynamics in coarctation of the aorta. In the majority of our patients the data do not support the hypothesis that humoral substances from ischemic kidneys are responsible for the systemic arterial hypertension accompanying these conditions.

89. FURTHER OBSERVATIONS ON THE GASTRIC CYTOLOGY OF PERNICIOUS ANEMIA

MELVIN I. KLAYMAN, M.D., AND BARBARA W. MASSEY, A.B., CHICAGO, ILL.

(INTRODUCED BY JOSEPH B. KIRSNER, M.D.)

Specific morphologic changes in squamous cells from the esophagus and columnar cells from the stomach in patients with *untreated* pernicious anemia, as reported by Graham and Rheault, include increase in cell size, multinucleation and altered chromatin distribution; the changes are reversible with appropriate treatment. Gastric columnar cells with greatly enlarged nuclei and folded nuclear membranes were seen by Massey and Rubin in patients with pernicious anemia *in remission* and were present as long as twenty years after adequate treatment.

In the present study, fifty-two patients with achlorhydria were examined for the presence of these cytologic changes. Thirteen of the group were proved cases of pernicious anemia in relapse; in ten instances both the squamous and columnar cell changes were apparent. Two cases showed aberrations only in the columnar cells; in one the changes involved only the squamous cells. In all instances, the altered squamous cells disappeared with treatment but the "P.A." columnar cells persisted.

Thirty-nine patients had no evidence of pernicious anemia. Gastric columnar cells characteristic of pernicious anemia were seen in ten of these patients; the squamous cells, however, were normal.

"P.A." squamous cells were seen twice in patients without pernicious anemia, both times occurring in cases of carcinoma of the esophagus.

Specific changes in *both* squamous and columnar cells appear to be characteristic of pernicious anemia in relapse. The finding of "P.A." columnar cells alone is more difficult to interpret. Their occurrence only in patients with gastric achlorhydria (with or without pernicious anemia) suggests an abnormality in the gastric mucosa which merits further study.

90. THE EFFECT OF SODIUM AMYTAL ON CIRCULATORY DYNAMICS IN PATIENTS WITH HYPERTENSION

ROSS C. KORY, M.D., AND ROBERT E. MABE, M.D., WOOD, WIS.

(INTRODUCED BY MAURICE HARDGROVE, M.D.)

The hypotensive effect of Sodium Amytal in patients with hypertension has been related clinically to the lability of the blood pressure in the "Sodium Amytal test." This test is predicated on the concept that the hypotensive

effect reflects arteriolar dilatation, although little supporting experimental data exist. A recent ballistocardiographic study suggested that Sodium Amytal lowers blood pressure primarily by decreasing cardiac output and that the total peripheral resistance may actually increase.

In the present investigation 14 male patients with well-established hypertension were studied by cardiac catheterization. Brachial and pulmonary artery pressures were recorded continuously and direct Fick cardiac output determinations were done in the resting postabsorption state and again 20 and 40 minutes after induction of light sleep with intravenous Sodium Amytal.

Both systolic and diastolic pressures decreased in all patients. The average fall in mean arterial pressure was 30.5 mm. Hg at 20 minutes and 34.5 mm. Hg 20 minutes after the Sodium Amytal. The cardiac output responded inconsistently, a slight decrease occurring in six patients, an increase in four, and no change in the remaining four patients.

The total peripheral resistance fell in 11 of the 14 patients and showed a definite increase in only one. In two patients peripheral resistance increased at 20 minutes but decreased at 40 minutes. The average over-all decrease in total peripheral resistance was 9.6 per cent at 20 minutes and 17 per cent at 40 minutes.

The arterial oxygen saturation showed a consistent fall of approximately 5 per cent by 20 minutes with no further change in the level at 40 minutes. Both minute ventilation and oxygen consumption decreased after Sodium Amytal in most instances.

It is concluded that in patients with hypertension intravenous Sodium Amytal, in doses sufficient to induce a light sleep, exhibits the following hemodynamic effects: decreases in arterial blood pressure, total peripheral resistance, minute ventilation, oxygen consumption, and arterial oxygen saturation, with variable changes in cardiac output.

91. MULTIPLE MYELOMA—CORRELATION BETWEEN THE MARROW AND THE CLINICAL AND ELECTROPHORETIC FINDINGS

C. K. Kubota, M.D. (By Invitation), Steven O. Schwartz, M.D., and Frank C. Putnam, Ph.D. (By Invitation), Chicago, Ill.

In the study of 78 cases of multiple myeloma, correlation was attempted between the marrow and the clinical and electrophoretic findings. The plasma cells of the marrow were classified into five groups, depending on the apparent age of the cells, and clinical and electrophoretic correlations were sought within each group.

The number of cases does not allow a statistically valid breakdown of the frequency with which each group is found, but in the present series the myelomas made up of fairly mature and mature cells predominated.

Findings.—(a) Because of the extreme variability in the clinical manifestations of multiple myeloma, pin pointing the onset of the disease is impossible. For this reason longevity figures are unreliable. Nonetheless, the patients who had more mature cells seemed to survive longer. (b) Patients who had a predominance of immature cells had the more profound anemias. (c) In patients whose myeloma was characterized by mature cells the anemia became progressively more severe. (d) No correlation was demonstrable between the extensiveness of marrow involvement and the cell types. (e) Forty cases showed evidence of renal involvement; seven of these had Bence Jones pro-

teinuria. No relationship to cell type was noted in this group or in others having Bence Jones proteinuria. (f) Of 40 cases studied by electrophoresis the preponderant protein was of the gamma type in 13, of the beta type in five, and of the "myeloma" type in four. In eight cases there was a diffuse increment in protein, in eight it was normal, and in one each the alpha 1 and alpha 2 proteins were increased. No correlation was seen between the cell type and the nature of the protein. (g) Hyperglobulinemia, whether made up of macroglobulins or euglobulins, did not characterize any cell type; nor did hyperglobulinemia correlate with true Bence Jones proteinuria. (h) Normal electrophoretic patterns with or without Bence Jones proteinuria could be found with any of the cell types. (i) No evidence was elicited that the electrophoretic pattern served any prognostic purpose or cast light on the duration or dissemination of the disease.

92. DRUG THERAPY OF AUTONOMIC HYPERREFLEXIA IN PATIENTS WITH SPINAL CORD LESIONS

N. B. KURNICK, M.D., LOS ANGELES, CALIF.

Patients with injuries to the cervical and upper thoracic spinal cord frequently demonstrate autonomic hyperreflexia. This is analogous to the skeletal motor hyperreflexia observed after cord section. The stimuli for the autonomic hyperreflexia may be cutaneous or visceral in origin. Stimuli administered to the rectum, urethra, and bladder are particularly effective. The manifestations are marked diaphoresis and severe, paroxysmal hypertension with associated symptoms of headache and flushing. Fatal cerebral hemorrhage has been observed. If the stimulus arises in the bladder, spasm of that organ is common. The effects of the ganglionic blocking agent, hexamethonium, and the adrenolytic agent, regitine, were investigated in 13 patients with cervical cord injuries.

The effects of the drugs were followed by the responses to cold pressor tests, physiologic filling of the bladder by urinary flow, rapid filling of the bladder through a catheter, and by the symptoms noted by the patient during routine care before and after medication. For acute tests, intravenous medication was administered, while prolonged therapy was per oram.

Regitine was uncertain in its action, occasionally reducing the response to stimuli; but more commonly, it was without apparent effect. Hexamethonium was uniformly useful in obliterating diaphoresis, reducing the hypertension which occurred on immersion of the lower extremity in ice water, and reducing bladder spasm. The effect on hypertension due to bladder filling and rectal stimulation varied from slight to complete suppression of the hypertension. Cystometric curves with simultaneous blood pressure observations which demonstrate the reduction in motor activity in the bladder musculature and suppression of hypertension, following intravenous hexamethonium, but not after regitine, will be shown. The usual side effects produced by the drugs were observed. Of these only postural hypotension, commonly present spontaneously in these subjects, was troublesome.

It may be noted that the hypertension of autonomic hyperreflexia is associated with bradycardia. The pathways involved in the hypertension, including the interrupted vasomotor depressor pathways, and the intact aortic and carotid sinus to cardiac depressor pathway will be illustrated. The effects of drug therapy and sacral neurotomy or cord destruction by alcohol will be compared.

93. CORRELATION OF SERUM MUCOPROTEIN AND GAMMA GLOBULIN IN VARIOUS DISEASE STATES

DANIEL S. KUSHNER, M.D. (BY INVITATION), HATTIE DYNIEWICZ, PH.D. (BY INVITATION), ALVIN DUBIN, M.S. (BY INVITATION), AND HANS POPPER, M.D., CHICAGO, ILL.

Under the tentative assumption that serum mucoprotein elevation reflects connective tissue alteration, and that serum gamma globulin elevation reflects reticuloendothelial reaction, and that both depend on the status of the hepatic parenchyma, an attempt was made to determine in 374 patients the diagnostic value of correlation of serum mucoprotein and gamma globulin levels, both determined by simple turbidimetric methods of de la Huerga.

The combination of low mucoprotein and low gamma globulin occurred only in myeloma. Low mucoprotein with high gamma globulin occurred in chronic hepatic disease, myeloma, and thyrotoxicosis. Subacute and chronic hepatic parenchymal disease was invariably associated with elevation of gamma globulin; however, half showed low and half normal or high mucoprotein. Elevated mucoprotein was encountered in fatty liver, florid cirrhosis, and Laennec's cirrhosis with infection. In contrast, hepatic metastases and extrahepatic biliary obstruction frequently showed normal gamma globulin with high mucoprotein levels.

Gamma globulin was elevated in most rheumatic diseases; simultaneous mucoprotein values were frequently normal in lupus, scleroderma, and convalescent rheumatic fever. Active rheumatic fever and rheumatoid arthritis were invariably associated with high mucoprotein, which in the defervescent period was more prompt to return to normal than the gamma globulin.

Gamma globulin and mucoprotein were frequently both elevated in tuberculosis and acute infectious disease. In contrast, one-third of neoplastic disease showed normal gamma globulin in the presence of high mucoprotein. Conversely, in a variety of cardiovascular and miscellaneous diseases, mucoprotein was normal in the presence of normal or high gamma globulin. The combination of normal mucoprotein and normal gamma globulin occurred rarely in systemic or chronic disease and in only 10 per cent of all patients examined.

The combination of serum mucoprotein and gamma globulin determinations appears, on the basis of the material studied thus far, to be of limited diagnostic utility with regard to a specific disease but of value in (a) excluding malignant disease, (b) differential diagnosis of jaundice, and (c) following activity of chronic infectious, rheumatic, and hepatic disease. The mucoprotein seems more specific and less sensitive, the gamma globulin less specific and more sensitive.

94. SERUM MUCOPROTEIN AND PAPER ELECTROPHORETIC PATTERNS IN MYELOMA

DANIEL S. KUSHNER, M.D. (BY INVITATION), ELAINE HERZOG, M.S. (BY INVITATION), ALVIN DUBIN, M.S. (BY INVITATION), AND HANS POPPER, M.D., CHICAGO, ILL.

Extremes of serum mucoprotein levels are encountered in patients with multiple myeloma, ranging from markedly depressed to extremely elevated values. An attempt was made to correlate these abnormalities with changes in the serum proteins determined by paper electrophoresis and the level of gamma globulin determined turbidimetrically.

In 20 patients with proved myeloma, serum mucoprotein level was low in one-third, normal in one-third, and high in one-third. Six elevated mucoprotein levels were associated with elevation of chemical gamma globulin, two with low gamma globulin. However, five instances of normal or low mucoprotein levels occurred with high chemical gamma globulin.

There was also no correlation between mucoprotein abnormality and dominant electrophoretic abnormality. The latter consisted of an abnormal protein with mobility between that of beta and gamma globulin, corresponding to the "M" fraction, in two-thirds (12) of the cases. Six others showed an abnormal protein with the velocity of gamma globulin, and one that of beta globulin. All six low mucoprotein levels occurred in the presence of the "M" protein electrophoretically. The high mucoprotein levels were not in any instance reflected in an abnormal protein migrating with the mobility of alpha globulin. Minor electrophoretic abnormalities in eight instances consisted of an increase in $alpha_1$ or $alpha_2$ fractions; in one the mucoprotein level was low, in three normal, and in four the values were high.

Elevation of turbidimetric gamma globulin occurred in 7 of 11 instances in the presence of "M" fraction electrophoretically.

Therefore no correlation was found among mucoprotein, turbidimetric gamma globulin, and abnormal electrophoretic proteins. With paper electrophoresis the "M" fraction is apparently demonstrated more frequently than with free electrophoresis, and may be reflected in the turbidimetric gamma globulin.

95. MYOCARDIAL BLOOD FLOW AND METABOLISM IN NORMAL AND HYPERTHYROID SUBJECTS

LEONARD LEIGHT, M.D. (BY INVITATION), VALENTINO DEFAZIO, M.D. (BY INVITATION), FREDERICK N. TALMERS, M.D. (BY INVITATION), TIMOTHY J. REGAN, M.D. (BY INVITATION), AND HARPER K. HELLEMS, M.D.,
DETROIT, MICH.

Utilizing the technique of coronary sinus catheterization, the myocardial metabolism of oxygen, glucose, lactate, and pyruvate has been studied in 14 patients with hyperthyroidism and 17 normal subjects to determine if alterations in myocardial metabolism might be responsible for the cardiac abnormalities frequently seen in patients with excess thyroxin production.

In both the hyperthyroid and the normal subjects there was marked variation in the myocardial extraction, varying from none to significant quantities of glucose, lactate and pyruvate. There was no statistical difference in utilization of these substances between the two groups, and there was no relation between the arterial blood concentration of the individual substrates and their extraction by the myocardium. Myocardial oxygen extraction was similar in the two groups, being 10.8 ± 2.9 volumes per cent in the normals and 9.5 ± 0.75 per cent in the hyperthyroid patients.

Using the nitrous oxide method, coronary flow was determined in eight of the normal and five of the hyperthyroid subjects. The coronary flow averaged 94 ml./100 Gm./min. (range 62 to 117 ml./100 Gm./min.) in the normals, and 141 ml./100 Gm./min. (range 106 to 140 ml./100 Gm./min.) in the hyperthyroid patients, representing, respectively, an average of 1.70 per cent and 1.32 per cent of the simultaneously observed cardiac output. The myocardial con-

sumption of oxygen in the normal patients was 9.7 ml./100 Gm./min. (range 6.6 to 13.0 ml./100 Gm./min.), while in the hyperthyroid patients it was increased to 12.1 ml./100 Gm./min. (range 10.0 to 13.5 ml./100 Gm./min.), accounting, respectively, for 4.60 and 4.41 per cent of the simultaneously observed total body oxygen consumption. In neither group was there a relationship between coronary flow or myocardial oxygen consumption and the cardiac output or left ventricular work.

It is concluded that (1) excess thyroxin induces an increase in myocardial oxygen utilization which is supplied by a simultaneous increase in coronary flow; and (2) the heart in hyperthyroidism appears able to metabolize glucose, lactate and pyruvate in a normal manner.

96. REGURGITATION TYPE JAUNDICE ASSOCIATED WITH CHLORPROMAZINE ("THORAZINE," SKF 2601-A) ADMINISTRATION

Rosaire E. Lemire and Robert A. Mitchell, Omaha, Neb.

(Introduced by F. Lowell Dunn, M.D.)

Brief mention has been made in the literature of a mild transitory jaundice appearing during Chlorpromazine therapy. In addition there are five cases, details of which have not been published, of a prolonged regurgitation type of jaundice.

We have observed three cases of persistent jaundice in elderly patients receiving Chlorpromazine. None had a history of biliary tract obstruction. The onset of the icterus was from 15 to 32 days after therapy was instituted, with a dosage range from 50 to 200 mg. daily. The duration of clinical jaundice was 66, 44, and 40 days, respectively, after withdrawal. All had severe pruritus. Chills, fever, pain, anorexia, nausea, vomiting, or malaise was not present. No hematological changes were noted except for a transient eosinophilia up to 7 and 17 per cent in two patients, respectively.

Liver function tests in these patients revealed a hyperbilirubinemia to 35.4 mg. per cent with biliuria, an increased alkaline phosphatase varying from 17.2 to 35.8 Bodansky units, and a normal cephalin flocculation. The cholesterol determination was normal in two, and elevated in the third to 584 mg. per cent, the cholesterol esters being 48 per cent. The thymol turbidity performed in two was normal.

All three patients underwent laparotomy. Two had no evidence of extrahepatic obstruction. The third, also with painless jaundice, had slight distention of the common bile duct with a partial stricture of the distal duct but free bile was present within the duodenum. In the gall bladder a single calculus 2 cm. in diameter was found. Liver biopsies taken from the latter patient and from one of the others showed similar changes consisting of plugging of the bile canaliculi with bile. The surrounding parenchymal cells showed brownish pigment deposition, otherwise they were normal. There were areas of spotty infiltration of inflammatory cells within the lobule. Examination of the portal areas revealed no evidence of obstruction or distention of the larger biliary channels. Photomicrographs will be shown.

These three patients are considered to represent hepatic toxicity to Chlorpromazine in two and a probable toxic reaction in the third. The mechanism of this complication is not known at this time.

97. EFFECT OF SOME CARBOHYDRASES ON HEMAGGLUTINATION—THEIR POSSIBLE ROLE IN SOME IMMUNOHEMATOLOGIC DISORDERS

ELIE P. LEROY, M.D. (BY INVITATION), WILMA SPURRIER, M.T. (A.S.C.P.) (BY INVITATION), CHICAGO, ILL.

(INTRODUCED BY STEVEN O. SCHWARTZ, M.D.)

In some cases of acquired hemolytic anemia the patient's erythrocytes are agglutinated by autoantibodies in higher titer than other erythrocytes. After splenectomy there is a gradual disappearance of antibodies from the serum of some patients. Since the spleen is a rich source of β-glucuronidase, this enzyme may affect agglutination of erythrocytes by antibodies.

Titrations of sera containing antibodies were carried out using erythrocytes previously treated for twenty minutes at 37° C. with suspensions of β-glucuronidase in normal inactivated AB serum. As controls, untreated erythrocytes and erythrocytes modified with suspensions of hyaluronidase, lysozyme and proteolytic enzymes in inactivated AB serum were used.

In serum or in bovine albumin medium, erythrocytes treated with β-glucuronidase in concentration of 20 mg. per cent or with hyaluronidase in concentration of 500 mg. per cent were agglutinated by specific antibodies in higher titer than untreated erythrocytes or erythrocytes treated with serum suspensions of the other enzymes. The titers of autoantibodies at 37° C. were also higher when tested with erythrocytes treated with serum suspensions of β-glucuronidase or hyaluronidase. Erythrocytes treated with these two enzymes gave a positive Coombs' test after two hours' incubation at 37° C. with sera containing high titer of cold agglutinins only. Hemagglutination was strongly inhibited by cortisone acetate in concentration of 20 mg. per cent, glucuronolactone and D-glucoascorbic acid in a concentration of 1 per cent; these compounds are also known inhibitors of β-glucuronidase.

Since β-glucuronidase and hyaluronidase are elaborated by many bacteria, and since some cancerous growths have high β-glucuronidase activity, these enzymes may affect the agglutinability of erythrocytes by antibodies in vivo.

In some infections and in some neoplastic diseases the titer of cold agglutinins is frequently increased; these autoantibodies which usually are more active at low temperature, may become active at 37° C. against erythrocytes previously exposed to elevated concentrations of β-glucuronidase or hyaluronidase in the tissues or organs affected.

The inhibition of hemagglutination by glucuronolactone and D-glucoascorbic acid could have therapeutic applications in immunohematology.

98. HEMOLYTIC PROPERTY OF SOME CARBOHYDRASES

THEIR POSSIBLE ROLE IN THE PATHOGENESIS OF SOME HEMOLYTIC ANEMIAS

ELIE P. LEROY, M.D., AND WILMA SPURRIER, M.T. (A.S.C.P.), CHICAGO, ILL.

(INTRODUCED BY STEVEN O. SCHWARTZ, M.D.)

Erythrocyte destruction depends on the balance between lysins which are normally present in the blood and tissues, and their inhibitors. Since red cells contain carbohydrates, carbohydrate-splitting enzymes may affect their life span.

The rate of hemolysis of erythrocytes suspended in concentrations of carbohydrases in inactivated AB serum was followed. Equal volume of packed red cells was added to enzyme suspensions at 37° C.; free hemoglobin in the supernatant was measured at various intervals after centrifugation.

β-glucuronidase and β-glucosidase in concentrations of 20 and 200 mg. per cent, respectively, caused complete hemolysis after 24 hours; hyaluronidase and lysozyme in concentration of 500 mg. per cent produced 50 and 30 per cent hemolysis, respectively. β-amylase in concentration of 500 mg. per cent was nonlytic. Erythrocytes left in some of the enzyme suspensions for only 20 minutes and resuspended in normal AB serum were partly hemolyzed after 24 hours. In serum containing antibodies against specific erythrocytes, β-glucuronidase and hyaluronidase in concentrations which were not hemolytic in normal AB serum, produced significant hemolysis; sera with autoantibodies from selected cases of hemolytic anemia gave similar results. β-glucuronidase hemolysis was inhibited by cortisone, silver nitrate, glucuronolactone, and D-glucoascorbic acid. All these substances are β-glucuronidase inhibitors.

Some of the lytic carbohydrases together with their inhibitors are found in normal serum and tissues. It is conceivable that any alteration in the balance between these enzymes and their inhibitors might cause excessive erythrocyte destruction. This may be the pathogenic mechanism of hemolytic anemia in neoplastic disease since cancerous tissues have increased glycolytic activity and this can affect the erythrocytes circulating in these tissues.

Since only antibody-sensitized erythrocytes were lysed by β-glucuronidase in physiologic concentrations, it is possible that this enzyme, which is abundantly present in the spleen, plays some role in immune hemolysis. The inhibition of the hemolytic action of β-glucuronidase by β-glucuronidase inhibitors suggest further chemotherapeutic approaches of some hemolytic anemias.

99. THE RATE OF CHOLESTEROL SYNTHESIS IN PATIENTS WITH MYXEDEMA

GEORGE V. LEROY, M.D., DELBERT M. BERGENSTAL, M.D., AND JON J. KABARA, M.S. (BY INVITATION), CHICAGO, ILL.

In the course of the development of a clinical laboratory procedure using carbon-14-labeled acetate to estimate the rate of hepatic synthesis of cholesterol, two patients with myxedema were studied. The amount of labeled cholesterol produced was so small that it was barely detectable in the plasma during the period of observation. Further studies of patients with myxedema are reported in this paper. Six patients had spontaneous myxedema and tests were performed before and during replacement therapy. Three patients had myxedema resulting from irradiation of the gland with x-rays or radioiodine. Four patients were studied before and after myxedema was induced with radioiodine. In every case of clinical myxedema, there was a striking reduction in the rate of cholesterol synthesis. The highest value obtained for the specific activity of plasma-free cholesterol after the intravenous dose of acetate did not exceed 20 per cent of the peak value observed in euthyroid subjects. In most instances labeled cholesterol was just barely measurable with the method used. The administration of thyroxin or desiccated thyroid had a prompt influence on the capacity to form labeled cholesterol, and normal specific activity-time curves were obtained well in advance of full clinical remission.

Since the plasma cholesterol level is elevated in myxedema, the size of the miscible pool of cholesterol was measured and the rate of turnover estimated in three cases. The low values for synthesis in myxedema were not the result of dilution alone, but appeared to reflect accurately the reduced rate of turnover. With the data available at this time it is not possible to say whether there is a parallel reduction in utilization *and* synthesis, or whether the low rate of synthesis may be dependent upon an altered homeostasis secondary to the defective utilization or excretion of the steroid in the athyroid subject.

100. THE UTILIZATION OF EXOGENOUS CHOLESTEROL FOR THE FORMATION OF ADRENAL CORTICAL HORMONES IN MAN

GEORGE V. LEROY, M.D., HAROLD WERBIN, PH.D. (BY INVITATION), M. EDWARD DAVIS, M.D., JURGEN E. PLOTZ, M.D., (BY INVITATION), AND DELBERT M. BERGENSTAL, M.D., CHICAGO, ILL.

On theoretical grounds it has been assumed that cholesterol of dietary, hepatic or adrenal origin can be used by the human adrenal cortex as a precursor for the production of cortical steroid hormones. Several groups of workers have reported the recovery of labeled 17-ketosteroids from the urines of subjects who were given labeled cholesterol or labeled acetate orally or intravenously. Previous studies from our laboratory have shown that the human adrenal in vivo can utilize either cholesterol or acetate for the production of steroids, the metabolites of which appear in the urine as 17-ketosteroids. The present paper is a report of the recovery and identification of two important 11-oxy-corticoids: tetrahydrocortisone-E, and 11-kete-etiocholanolone, from the urines of patients who were given labeled cholesterol orally or intravenously. It is generally accepted that these two corticoids are metabolites of steroid hormones produced solely by the adrenal cortex. In the first experiment, the precursor cholesterol labeled with tritium was fed for several days before urine was collected. In the second experiment, a mixture of tritium-labeled and carbon-14-labeled cholesterol was given intravenously and urine was collected for the following 48 hours. The urine samples were subjected to acid hydrolysis and beta-glucuronidase hydrolysis, respectively. The appropriate fractions were chromatographed and a small amount of each metabolite was obtained. Pure "cold" samples of each steroid were then used as a carrier to obtain crystals for identification and for radioassay by isotope dilution analysis. Samples of blood were obtained during the urine collection period, and the specific activity of the plasma-free cholesterol was determined. In each case, the values for specific activity of the metabolite were lower than the values for the specific activity of the plasma cholesterol. These results demonstrate that exogenous cholesterol is a precursor for at least two adrenal cortical hormones. It appears evident from this study that there is no need to assume the existence of an active form of cholesterol as precursor for cortical hormones since ordinary cholesterol can serve that purpose.

101. LONG-TERM EFFECT OF X-RAY IRRADIATION ON ACID SECRETION IN PATIENTS WITH PEPTIC ULCER

ERWIN LEVIN, M.D., JOSEPH B. KIRSNER, M.D., WALTER L. PALMER, M.D., AND CHARLES B. CLAYMAN, M.D. (BY INVITATION), CHICAGO, ILL.

The long-term effect of small quantities of x-ray irradiation to the body and fundus of the stomach upon the secretion of hydrochloric acid was studied in a group of approximately 1,200 patients, almost all of whom had duodenal

ulcer. The morning basal gastric secretion and the response to the histamine analog, histalog (3-beta aminoethylpyrazole), were determined before therapy and at varying intervals totaling one to five years after irradiation.

Immediately following therapy, the volume of gastric content and output of hydrochloric acid in the basal secretion decreased significantly in 80 per cent of patients. In addition, the pattern of basal secretion was converted from a continuous output of acid characteristic of duodenal ulcer, to an intermittent secretion more often observed in patients without duodenal ulcer. In some individuals the suppression of gastric secretion was brief, disappearing within three to nine months. However, in approximately 60 per cent of cases the inhibitory effect persisted and was demonstrable three to five years after therapy had been concluded.

Similar results were observed in the response to histalog, although acidity in occasional specimens of gastric content reached pretreatment levels. Persistent achlorhydria developed in approximately 7 per cent of the series after irradiation.

The development of irradiation-anacidity invariably resulted in complete healing of peptic ulcer, with no recurrences for the duration of the achlorhydria. Reduction in acid output and conversion from continuous to intermittent secretion after therapy usually decreased the incidence and severity of recurrences. Return of peptic ulcer was preceded by a return of acid secretion to preirradiation levels.

102. TISSUE BINDING OF HYDROCORTISONE

MARVIN E. LEVIN, M.D. (BY INVITATION), AND WILLIAM H. DAUGHADAY, M.D.,
ST. LOUIS, MO.

Hydrocortisone, when administered intravenously into rats, disappears more rapidly than can be accounted for by simple diffusion into various compartments of body water. After 15 minutes only 2 per cent of a given dose remains within the blood. The factors which might contribute to this rapid rate of disappearance have been studied.

In vitro experiments have been carried out in which rat tissue slices have been incubated with hydrocortisone. The amount of hydrocortisone which remained in the incubation medium was compared with that which could be recovered when the tissue slices were included in a vigorous extraction procedure. Evidence was obtained that steroid (Porter-Silber reactive material) left the media to combine with the tissue slices. Binding could also be demonstrated when tissue slices were removed from the media at the end of incubation and were washed repeatedly with cold buffer free of steroid.

Hydrocortisone combines rapidly with tissues. Rat liver slices incubated for five minutes in a medium containing 167 μg of hydrocortisone per milliliter bound 0.2 μg of steroid per milligram of tissue at which time little chemical degradation of hydrocortisone had taken place. The amount of bound steroid remained relatively constant for incubation periods as long as three hours.

Hydrocortisone binding by liver slices was proportional to its concentration in the medium. The combination of steroid with tissue occurred at 0°. At this temperature the ability to degrade hydrocortisone was markedly inhibited. The affinity for hydrocortisone of various rat tissue slices was found to be: liver > diaphragm > kidney > spleen > fat. The degree of binding was not correlated with the ability of the tissue to degrade hydrocortisone.

It is suggested that hydrocortisone combines with tissues by a rapid, nonenzymatic mechanism which precedes degradation. The affinity of tissues for hydrocortisone may contribute to the rapid disappearance of steroid from the blood and may be of general physiologic significance.

103. THE INFLUENCE OF SEVERE BURNS ON PROTEIN METABOLISM AS MEASURED BY UPTAKE AND EXCRETION OF L-METHIONINE LABELED WITH RADIOSULFUR

WILLIAM C. LEVIN, M.D., JOHN E. PERRY (BY INVITATION), T. G. BLOCKER, JR., M.D. (BY INVITATION), AND S. R. LEWIS, M.D. (BY INVITATION), GALVESTON, TEX.

It is well established that severe burns produce extreme alterations of protein metabolism, but methods used in the investigation of this problem have not been precise enough to permit adequate evaluation of both anabolic and catabolic rates. The present report involves the use of an isotopic method.

Four normal patients, four nutritionally depleted nonburn patients, and five severely burned patients are included. L-Methionine labeled with S-35 was administered intravenously to each patient. Rates of incorporation within the serum proteins, rates of loss of activity from the serum proteins, and urinary excretory rates were measured.

In the normal and depleted patients, the peak of activity of the serum proteins varied from 6 to 17.3 per cent of the administered dose. In the severely burned group, the peak activity was 20.6 to 48.5 per cent. Urinary excretion of radioactivity appeared to correlate directly with the peak of serum protein activity. In the normal and the depleted group, urinary excretion during the first 24 hours varied from 4.2 to 12.6 per cent. Renal excretion in the first 24 hours was 28.5 per cent in one mild burn and 31.9 per cent in one severely burned patient. We have previously reported that the rate of decrease of activity in the serum proteins is much greater in the severely burned than in normal patients. This observation was confirmed in the present study.

The rapid disappearance of activity from serum proteins in the burned patient as well as the increase in rate of renal excretion have been interpreted to be manifestations of an increased rate of protein catabolism. In addition to this, however, the burned patients also show a much greater quantitative uptake of radioactivity within the serum proteins during the first 24 hours. We have interpreted this to be a manifestation of an increased rate of synthesis of serum proteins.

This suggests that the nutritional defect in the burned patients encompasses an increased rate of protein anabolism as well as an increased rate of protein catabolism. It may further indicate that burned patients can utilize large amounts of ingested protein provided alimentation is not impaired.